Patch selection behaviour in the presence of environmental constraints

by

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Abstract

Habitat selection behaviour is the primary way in which organisms are able to regulate encounters with their biotic and abiotic environment. An individual chooses an area that best meets their current needs, particularly regarding safety and the presence of high-quality food. Several physical aspects of the environment can make it difficult for individuals to assess the relative habitat quality of the areas available, thus leading to sub-optimal habitat selection. In this thesis, I investigated the way in which two aquatic habitat constraints - obstacles to movement between patches and turbidity - affected the ability of fish to make optimal patch choices, using threespine stickleback *Gasterosteus aculeatus* as a model species. Laboratory experiments showed that when movement between patches was hindered by increasingly challenging obstacles, groups of stickleback did not move as freely between the patches, and thus had greater deviations from the predictions of the Ideal Free Distribution (IFD). I also demonstrated that, unlike other species, stickleback do not use turbid environments to avoid predator detection. A trend was seen towards avoidance of a turbid food patch regardless of risk level, although this was not statistically significant. As expected, the stickleback avoided feeding in the presence of a predator regardless of water clarity. Overall, I found that both turbidity and movement constraints can have significant impacts on patch use and distribution in the threespine stickleback. Both turbidity and ease of transit will impact the distribution of ecologically important species like the threespine stickleback, and therefore should be taken into account when studying habitat selection in the wild.

Dedication

To my family,

by blood and beyond.

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Co-Authorship Statement

Jennifer Labelle completed the research in this thesis and all written work under the guidance and supervision of Dr. Mark Abrahams. The contributions for the data chapters are as follows:

Chapter 2: Jennifer Labelle conceived the project with input from Mark Abrahams. Jennifer Labelle collected and analyzed the data, and wrote the chapter with Mark Abrahams' guidance and input.

Chapter 3: Mark Abrahams conceived and supervised the project. The experiment was adapted from one designed by Scott Chiu under Mark Abrahams' supervision. Jennifer Labelle collected and analyzed the data and wrote the chapter with guidance and input from Mark Abrahams.

Chapter 1
General Introduction

1.1 Physical constraints to habitat selection

Habitat selection is the most important and complicated behavioural decision animals make, as the selected habitat will impact all other interactions the animal has (Lima and Zollner 1996, Morris 2003). When selecting an ideal habitat, an individual must be able to collect accurate and up-to-date information on the quality of all available habitats, assess them against their current needs, and select the habitat that will maximize their current fitness (Roever et al. 2014). In other words, they must select a habitat that gives them the best opportunities to survive and reproduce.

The specific biotic and abiotic characteristics that constitute an 'ideal' habitat differ among species, conspecifics and even within the same individual, depending on both ontogeny (Werner and Hall 1974, Rachlow and Bowyer 1998), and current priorities (Roever et al. 2014). Day-to-day survival, however, depends most strongly on avoiding predation and acquiring sufficient high-quality food (DeCesare et al. 2014). Being eaten is an instantaneous end to an animal's ability to reproduce, and insufficient energy also makes survival and reproduction difficult to impossible. I therefore narrowed the focus of this thesis towards habitat selection behaviour based only on food acquisition and predator avoidance.

Food abundance and safety are often inversely related (Heithaus and Dill 2002). Areas that contain an abundance of food often draw a large population of individuals. This attracts a large number of predators, and safety becomes compromised (Chiu 2006).

Areas with low food abundance, or low-quality food, draw fewer individuals, which then attracts fewer predators (Nielsen et al. 2006). Individuals therefore often face a risk tradeoff between safety and starvation, which has large effects on habitat use (Lima and Dill 1990).

Optimal habitat selection requires accurate assessment of the relative quality of potential habitats (Roever et al. 2014). However, it is rarely possible for an animal to collect perfect information regarding food abundance and predator presence location (Abrahams 1986, Koops and Abrahams 1998, Koops and Abrahams 2003), as there are several physical characteristics of the habitats that prevent such information collection (Koops and Abrahams 2003), such as habitat complexity and poor light penetration (James and Heck 1994). This can lead to animals making use of suboptimal habitats when better ones are available (Matsumura et al. 2010, DeCesare et al. 2014).

Increasing the effort necessary for individuals to move between patches or habitats can have significant impacts on their habitat use (Martin et al. 2008). Even on a small scale, when patches are more distant from one another or there are physical barriers that make travel between them difficult, the ability of animals to accurately assess differences in habitat suitability is affected, often leading groups to overuse poorly resourced patches and underuse rich ones (Korona 1990, Kennedy and Gray 1997). This concept is best exemplified by habitat fragmentation: when patches of usable habitat are separated by distance, roads, and other travel barriers, dispersal and migration may decline, leading to population die off from overcrowding and food shortages (Fahrig 2003, Haddad et al. 2015).

In aquatic habitats, the visual characteristics of the water can change dramatically, over both time and space (Orpin et al. 2004). Small sedimentary particles get stirred up into the water, creating turbidity. Turbidity, which has a veiling effect similar to that of fog, reduces the ability of aquatic animals to visualize their surroundings (Utne-Palm 2002). Most species of fish use vision to locate both their food and their predators, making it more difficult to feed and avoid predators in highly turbid waters. However, the ability of predators to capture their prey will also be compromised (de Robertis et al. 2000, Utne-Palm 2002), meaning that turbid environments are often less risky than clear environments. For this reason, many small fishes will use turbid environments to avoid detection by their predators, despite the potential reduction in food acquisition (Gregory 1993, Chiu and Abrahams 2010).

1.2 Goals of thesis and chapter structure

In this thesis, I investigated two potential habitat constraints in a laboratory setting – visual constraints and constraints to movement – and assessed the ability of threespine stickleback *Gasterosteus aculeatus* to make energy-maximizing habitat decisions under these circumstances. The magnitude to which these constraints impacted the ability of the stickleback to forage efficiently was assessed by measuring the deviations from the distributions predicted by the ideal free distribution (IFD) theory (Fretwell and Lucas 1970).

In Chapter 2 of this thesis, I investigated the impact of movement constraints on the ability of groups of ten stickleback to match food input. I first examined the feasibility of my apparatus to create travel challenge for this species. The apparatus constrained

travel through the use of clear barriers that altered the number of tunnels available for transit between two equal food patches in an aquarium. In the second experiment, I randomized the food ratio between two feeders, and assessed whether the fish were able to match resource input as travel became more difficult. In Chapter 3, I used a similar experimental design to Chiu and Abrahams (2010) to determine if threespine stickleback use turbidity to avoid predator detection, or if it was perceived as a constraint to food acquisition and therefore avoided. Finally, in Chapter 4, I discuss the general findings of the thesis and put my results into an ecological perspective.

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Chapter 2

The effect of travel costs on movement and distribution of stickleback between food patches

2.1 Abstract

The Ideal Free Distribution (IFD) theory, which predicts that a population of individuals will match the distribution of a patchily distributed resource, is widely used in ecology to explain the spatial distribution of animals. While many studies have shown general support of its habitat matching prediction, others have described a systematic pattern of undermatching, where too many animals feed at patches with fewer resources, and too few animals feed in richer patches. These results have been attributed to deviations from several of the assumptions of the IFD. One possible variable, the cost of travelling between patches, has been understudied in this regard. Here, I investigated the impact on resource matching when travel costs were manipulated in a simple laboratory experiment involving two continuous input patches. This setup allowed me to control for extraneous variables, and decouple time costs from energetic costs of travel. I found conformity to the hypothesis, where there were trends towards less movement between patches and greater discrepancies from the IFD predictions as the time cost of travel increased. However, the relationship is more complicated than hypothesized, as the best fit to the IFD occurred at intermediate travel costs.

2.2 Introduction

Habitat use and species interactions are largely determined by how a population is distributed in space and time. This, in turn, is largely dictated by the distribution of

resources (e.g. food, water, mates, etc.) and competitors (Tregenza 1994). Several theories have been proposed to predict the distribution of animal populations based on the location of their resources, but the most widely used and accepted is the Ideal Free Distribution (IFD) theory (Fretwell and Lucas 1970).

The IFD predicts that, when limited resources are distributed in discrete patches, animals disperse themselves to match the proportion of resources within each patch (Fretwell and Lucas 1970). In this situation, an individual's share will increase with greater resource density and will decrease with greater competition pressure. When each animal in a population follows this rule, their access to resources, and therefore their fitness, is maximized. Any individual that deviates from this distribution will get less, and thus it represents a stable (Nash) equilibrium (Milinski 1984). The IFD assumes that the animals are *ideal* in their knowledge of the distribution of resources (each individual knows where all the patches are located and the relative quality of each patch at all times) and *free* to feed at any patch (they are impeded neither by competitors, nor by travel costs).

These assumptions can be difficult to meet in realistic situations; nearly all animals have limited information and are variable in their competitive abilities. While there are many studies that support the predictions of the IFD despite deviations of some of the assumptions (Harper 1982, Godin and Keenleyside 1984, Milinski 1984, 1994, Regelmann 1984, Abrahams and Dill 1989, Kacelnik et al. 1992), a number of studies have demonstrated a consistent bias in patch use: individuals underuse rich patches and overuse poor patches (Abrahams 1986, Kennedy and Gray 1993, Earn and Johnstone 1997). This bias has been explained by violations of three main assumptions: limited

information (Abrahams 1986, Cartar and Abrahams 1997, Ranta et al. 1999), unequal competitive abilities (Parker and Sutherland 1986, Houston and McNamara 1988, Grand 1997), and varying levels of interference competition (Sutherland 1983).

Distribution bias has also been noted in several studies that investigated the effects of costly travel between food patches (Baum 1982, Regelmann 1984, Houston and McNamara 1987, Korona 1990, Kennedy and Gray 1997, Baum and Kraft 1998). However, the direction of deviation is variable (either overmatching or undermatching), and, theoretically, depends on which direction of travel is more costly (Åström 1994). Costs to travel can be incurred in a situation of patchily-distributed food in two main ways: the energetic cost of travelling from one point to another, which is negligible on the scale of patch choice (Schmidt-Nielsen 1972), and the time cost of forfeited feeding opportunities that take place while an individual is in transit (Milinski 1994). To make this situation more complicated, when patches are separated by distance or obstacles, there may be a confounding effect between travel cost and imperfect information (Roberts and Goldstone 2005); distance decreases the ability of animals to discriminate between patch profitability levels (Kennedy and Gray 1997).

In this paper, I considered the role of travel costs on the ability of threespine stickleback *Gasterosteus aculeatus* (hereafter referred to as stickleback) to match the resource distribution between two food patches. I manipulated travel costs without affecting the ability of the fish to assess patch quality, which allowed me to examine the impact of travel cost as an independent parameter. By increasing the difficulty level required for fish to move between two patches, I increased the time away from a food patch and therefore the amount of food forfeited by the fish that chose to move between

patches. The fish were trained to recognize a feeder bar as the only source of food, thus making the cost of searching negligible. If travel costs do impede the animals' movement between resource patches, I predicted that it would manifest itself on animal distribution by introducing inertia in their spatial distribution, and would ultimately result in a reduced conformity to the habitat matching prediction of the IFD that is proportional to the magnitude of the travel cost.

2.3 Methods

2.3.1 Stickleback collection and maintenance

Stickleback are commonly used in behavioural experiments due to their widespread distribution, ease of care, and general "hardiness" (Moran et al. 2010). They prefer to forage in shoals, and are more successful foragers in groups as well (Kendal et al. 2004). Stickleback are so highly invested in social foraging that they will ignore previous private information (information collected through their own experiences), instead basing their decisions on current public information (information collected from the cues of other members of the population) (Webster and Hart 2006). For this reason, when sticklebacks are unable to collect private information about the quality of multiple resource patches, they will choose to trust the collective decision and forage with the larger shoal (Milinski 1979, Gotceitas and Colgan 1991, Krause 1992). This causes a larger skew in the distribution as the larger shoal increases in size, making it apparent when the conditions of the IFD have not been met.

The stickleback were collected between February and October 2013 from Long Pond in St. John's, NL (47°34'34.19"N, 52°44'08.84"W) using krill-baited minnow traps

left overnight. The fish were transported to the Ocean Sciences Centre, where they were inspected visually for signs of parasitic infection by *Schistocephalus solidus*, as indicated by a large distended belly, and *Glugea anomala*, which causes large white cysts on the skin. Those with obvious infection were not used in experiments, as both parasites have been linked to abnormal foraging behaviour in stickleback (Milinski 1985). All fish were placed into identical 38 L glass holding aquaria in groups of 50-75. The tanks were maintained on a flow-through freshwater system from a communal header tank. Water in the header tank was aerated using an oxygen compressor bubbler, and individual tanks were outfitted with air bubblers. Dissolved oxygen levels were kept between 90-110% saturation, with some occasional fluctuations of ±20% outside this range. Fish were held in natural ambient light conditions and ambient temperatures, which ranged from 8 °C in the winter to 17 °C in the summer, and were fed *ad libitum* with TetraMin Tropical Fish Flakes and frozen brine shrimp *Artemia salina*. The fish were allowed to acclimatize to laboratory conditions for at least 2 weeks before being trained and used in trials.

Prior to use in each experiment, the fish were trained to navigate the apparatus and use the automated feeders used during trials (described below, page 13). Groups of approximately 50 fish were trained at a time, and all tunnels were open between the chambers during training. Throughout the training period, the stickleback were fed frozen brine shrimp twice daily using the automated feeders. The total amount of shrimp provided a maintenance ration of approximately 5% body of their body weight, divided equally between the two feeders during two feedings per day. The training period continued for 1 week, when fish were seen to consistently divide equally between the feeders, which indicated that they had learned to travel between the two chambers,

perceived the equal feeding opportunities they provided, and were following a spatial distribution consistent with the ideal free distribution. Any fish that displayed territorial or agonistic behaviour during the training period were removed and were not used during the experimental trials. Overall, fewer than 10 fish had to be removed from training groups for this reason.

2.3.2 Experimental apparatus design

Similar apparatuses were used for both experiments, and three identical apparatuses were built for each in order to run three feeding trials simultaneously. A 151 L tank was divided into two equal compartments that were separated by an 8 cm gap using clear acrylic glass sheets (Figure 2-1). The two end compartments were connected across the centre chamber by clear acrylic tunnels, each of which could be closed off at both ends by clear acrylic doors. These doors restricted movement between the two chambers without restricting visual information, which allowed us to assume that the fish would be 'ideal' in their knowledge of the food patch distribution as they could see both the food entering at the other end of the aquarium, and the activity of the fish at the second feeder. The two long sides of each aquarium were lined with black plastic sheets in order to minimize disturbance to the fish during experiments. Black plastic sheeting also surrounded the area containing the experimental set-up.

One automated feeder was placed in each compartment of the experimental apparatuses, only during feedings. These feeders, described in detail in Abrahams 1989, were designed to provide a continuous input, non-depleting food source for up to 45 minutes. A spreader bar that spanned the end of the tank made it difficult for individuals

to defend the food. This complied with the 'free' assumption of the IFD.

The two experiments described in this chapter required slightly different apparatus designs. For the first experiment, the apparatus had five tunnels arranged in a pentagon shape that connected the compartments, as shown in Figure 2-1. The doors covering either end of each tunnel attached using a single stainless-steel screw, and could pivot open and closed with minimal disturbance to the fish. This was done in order to easily alter the tunnel access for each feeding trial. For the second experiment, the chambers of the apparatus were connected via nine tunnels arranged in a 3x3 grid. The tunnel doors were fixed throughout the 5-day-long trials with each group in experiment 2, so the doors were screwed into place using two stainless-steel screws before the experiment began. For the first experiment, the apparatus was maintained on a flow-through system, allowing the water temperature to be maintained between 13°-15°C. The dissolved oxygen level during trials ranged from 95-109%. Issues with the freshwater system in the building during the second experiment caused us to switch to a standing water system, where the temperature ranged from 17°-22°C, and dissolved oxygen levels ranged between 70-85%.

Two CCTV cameras recorded the activity in each tank during trials. The two video signals were combined into a single split-screen recording using a Videonics MX-1 video mixer and recorded onto DVDs for later analysis. Combining the two images allowed me to view activity across the whole tank simultaneously. The positions of the cameras differed between the two experiments. In experiment 1, one camera was suspended above the tank to capture an overhead image of the entire tank, while the second camera was directed towards one end of the tank to obtain a head-on view. This

arrangement allowed an accurate count of the fish moving between chambers to be obtained, in addition to feeding activity at both feeders. In the second experiment, a camera was set up facing each end of the two chambers to allow accurate assessment of the number of fish feeding at the two spreader bars.

2.3.3 Experiment 1 – Effect of obstacles on movement of stickleback between food patches

2.3.3.1 Experimental protocol

Experiment 1 was carried out during May and June of 2013. One or two days before the experiment began, groups of 10 similarly sized fish (average length range within groups: 8.6 mm, maximum length range: 11.1 mm) were selected from the training group, anesthetized using 750 μL of 1:9 eugenol:ethanol solution dissolved in 1 L of water, and measured for total length and wet weight (Table 2-1). Once the fish regained normal activity levels after ~30-60 minutes of recovery, they were placed within the apparatus. Trained fish that were not used in a trial were retained to replace possible mortalities and to facilitate training in the following groups. In total, six groups of 10 fish were used for this experiment.

Each group of fish was tested under three travel cost manipulations: high (1 tunnel open, representing 0.98% of the total area), medium (3 tunnels open; 2.95% total area), and low travel costs (5 tunnels open; 4.92% total area). Each travel cost was replicated five times for a total of 15 trials per group. Treatment order and the configuration of open holes were randomized across the 15 feeding trials separately for each group. Feeding trials were conducted at approximately 0930, 1230 and 1530 for five consecutive days.

Approximately 30 minutes before the trial commenced, all acrylic doors were closed and the fish were moved by dip net to a starting distribution of 8:2 between the two chambers. The start chamber – the chamber with 8 fish at the beginning – was randomized to offset side bias. Feeding trials consisted of equal amounts of non-depleting food being delivered at equal rates to the two chambers by the automated feeders. Fifty whole, thawed brine shrimp were placed in each feeder with 1.5 L of water, which allowed the feeder to provide a continuous-input food source for the 25 minute long trial. This provided each group of fish with 300 brine shrimp per day, a feeding rate of approximately 10% of their body weight. Trials commenced when the appropriate tunnel doors were opened, feeders were started, and the video cameras began recording. Upon completion, after 25 minutes had passed, the brine shrimp remaining in the feeders were counted, and any uneaten shrimp within the apparatus were removed using a syphon. If at the end of the trial the feeders were found to have supplied unequal food to each chamber (>10 shrimp discrepancy between chambers), the trial was excluded from the final analysis and recompleted after the remaining scheduled trials. This issue arose in approximately 20% of the trials.

2.3.4 Measurements and Analysis

The movement and feeding behaviour of the fish during trials was determined from the recorded videos. Two measurements were collected from the recorded trials: the total number of trips between chambers (switch rate, per 25 minute trial), and the change in distribution. The total switch rate was a count of the times any fish crossed completely between the two sides, i.e. exited the opposite opening of the tunnel to which it entered,

over the course of the 25-minute long trial. Incomplete or partial crossings were not counted. Individual fish were not distinguishable, so I was unable to determine how many individual fish used the tunnels. To calculate the change in distribution, the number of fish feeding in each chamber was first determined at one-minute intervals throughout the trial. The number of fish feeding was determined by counting the number of fish seen feeding within 3 seconds before and after each minute mark of the video. A fish was presumed to be feeding "it was swimming in the close vicinity … of a feeder bar and oriented toward the feeder bar or seen intercepting food or picking up from the bottom" (Chiu 2006), or was seen accelerating quickly toward the feeder bar. The count of fish feeding was then converted into the proportion of fish feeding in the start chamber (fish feeding in start chamber/total fish feeding in both chambers). The mean of these values was then taken to obtain the average proportion, which was converted to a percentage. In open aquaria, stickleback reach a distribution predicted by resource distribution after 3 minutes (Milinski 1986), so the average proportion was calculated only from the values in minutes 4 to 25. The change in distribution was then calculated as the difference between the start distribution (i.e. 80%) and the percentage of fish that fed in the start chamber over the course of the trial.

For both measurements, the values were averaged across the five feeding trials for each group in order to obtain a single value per treatment to be used in the statistical analysis. Any groups where the participation rate was lower that 60% across the trials was removed from analysis. Participation was measured as the mean number of fish feeding throughout the trial, divided by the total number of fish (10). Both switch rate and change in distribution were analyzed using a Friedman's ANOVA in R version 3.0.

2.3.5 Experiment 2 – Effects of obstacles on the ability of stickleback to assess and match food distribution

2.3.5.1 Experimental Protocol

Using the same protocol as experiment 1, after the training was complete groups of 10 similarly sized fish (average length range within groups: 5.73 mm; maximum length range: 7.4 mm) were selected, measured (Table 2-2), and placed in the experimental apparatus upon recovery from anesthesia. Trials began the following day. Twelve groups of 10 fish were used for this experiment between October and December 2013.

Each group was randomly assigned to one travel cost treatment: high travel costs (3 tunnels open, representing 2.95% of the total area), medium travel costs (6 tunnels open; 5.91% total area), or low travel costs (9 tunnels open; 8.86% total area). The configuration of tunnels that were open was arranged so that the same number of tunnels was available within each row and column of the grid of tunnels. In other words, when three tunnels were open, there would be one tunnel open in each row and column. Within this arrangement, the open tunnels were randomized as much as possible. The doors were attached on the applicable tunnels the day before the trials began, when the fish were not in the apparatus.

Five food distributions were used to assess conformity to the predictions of the Ideal Free Distribution: 1:9, 3:7, 5:5, 7:3 and 9:1. The required distribution was achieved by dividing 100 whole frozen brine shrimp between the two feeders containing 1.5 L of water. The order in which the distributions were presented was randomized across 5 days. Three feeding trials took place each day at 0930, 1230 and 1530, with the same food

distribution provided at each feeding throughout the day. The starting distribution of stickleback was not manipulated for this experiment. Trials commenced when the feeders were started and the video began recording, and lasted 25 minutes. Upon completion of the feeding trial, the volume of water and number of brine shrimp remaining in the feeders was measured to ensure that the overall food delivery rate was suitable (i.e. the majority of the food had entered the apparatus during the feeding trial, in a distribution that approximated that of the treatment).

2.3.5.2 Measurements and Analysis

From the video recordings, the number of fish feeding within each chamber was documented every minute throughout the trial, as described above. The number of fish feeding in each chamber from minutes 4 to 25 was averaged into a single value for each trial. Four minutes was chosen as the cutoff as a previous study showed that threespine stickleback take approximately 3 minutes to reach an IFD distribution (Milinski 1979). The number of fish feeding in chamber A was then converted into a percentage of fish feeding using the equation:

$$Percentage\ of\ fish\ feeding = \frac{Fish\ feeding\ on\ side\ A}{Fish\ feeding\ on\ side\ A + Fish\ feeding\ on\ side\ B} \times 100$$

This value was analyzed using ANCOVA in which the travel cost treatment (number of tunnels available, as a categorical variable) was the explanatory variable and the food distribution was the covariate. Similarly to experiment 1, only groups that had a mean participation rate of 60% were included in the analysis.

The linear relationship between the fish distribution and the food distribution was analyzed for each travel cost using linear regression. In order to determine which travel

cost level resulted in the fish with the best fit to the predicted distribution (where x=y), I calculated the mean (± SE) slope and intercept for each travel level, and used t-tests to test both the slope and the intercept of each regression line against the predicted values of 1.0 and 0.0, respectively.

All analyses were completed in R version 3.0.

2.4 Results

2.4.1 Experiment 1 - Effect of obstacles on movement of stickleback between food patches

The six groups of stickleback used for experiment 1 were significantly different in both total length (ANOVA, $F_{5,54} = 60.10$, $p<0.0001$) and wet weight ($F_{5,54} = 80.99$, $p<0.0001$). While groups with higher body weights showed a trend toward being more active, the effect of mean group weight on travel (where travel was determined by the total sum of crossings across all 15 trials) was not significant (Spearman's rank correlation: $r_s=0.7143$, n=6, p=0.111). The summed absolute values of the change in distribution by group also were not correlated to the average wet weight of the groups (Spearman's rank correlation: $r_s=0.60$, n=6, p=0.280). Fish size was therefore not included in the analysis.

All 6 groups met the required 60% participation rate in each treatment (Table 2-3). Both the switch rate during each trial and the change in distribution were analyzed for outliers in the data. For switch rate data, outliers were defined as any value that increased the mean within the group/treatment condition by >2.5x.When compared with other techniques for defining outliers, such as values falling outside ± 2SD from the mean, as

for parametric data, this method was highly conservative. Three individual trials were removed from further analysis following this rule (one from group 1 – high cost travel, one from group 2 – medium cost travel, and one from group 5 – high cost travel). No data were removed for the analysis of the change in distribution measure, as no values were extreme compared to the expected change in distribution of 3 fish required to reach an equilibrium distribution.

The switching rate between the two chambers increased significantly as more tunnels became available (Friedman's ANOVA: $\chi^2 = 12.0$, df = 2, p = 0.002). While there was considerable variance across the groups, the highest mean number of tunnel crossings occurred for all groups when all five tunnels were open (Table 2-4, Figure 2-2). The relationship between the number of tunnels available and ease of transit appears to be non-linear: increasing from one tunnel to three tunnels resulted in a smaller increase in switching between chambers than did the increase from three tunnels to five tunnels.

The change in distribution (**Error! Reference source not found.**) was also ignificantly affected by the travel costs (Friedman's ANOVA: $\chi^2 = 8.33$, df = 2, p = 0.016), though the magnitude of change was less extreme than predicted by the IFD in all but three low-cost travel trials (a single trial for each of group 1, group 4, and group 5). In trials with the highest travel costs, the distribution rarely changed. In the trials with a low cost to travel, more fish moved to the chamber with fewer fish. If the relationship between the number of tunnels available and the number of fish switching to the second chamber continues linearly, linear regression indicates that approximately 12.6 tunnels (12.35% total area) would be required for three fish to switch, and thus match the food distribution.

2.4.2 Experiment 2 – Effects of obstacles on the ability of stickleback to assess and match food distribution

Two groups were removed from analysis due to low participation rates (Table 2-5). Group 1 (low travel cost) and group 2 (medium travel cost) had average participation rates of 23.5% and 33.5%, respectively. Consequentially, only three groups remained in the low and medium travel cost treatments for analysis.

The ten groups of stickleback used for experiment 2 were found to have statistically significant differences in both total length (ANOVA, $F_{11,105} = 39.04$, $p<0.0001$) and wet weight ($F_{11,108} = 41.48$, $p<0.0001$). In order to determine if size played a role in ability of the groups to match the predicted IFD, the sum of departures from the IFD predictions was calculated for each group. Using a two-factor ANOVA with weight and the travel cost level as predictors and the sum of departures as the explanatory variable, I found that mean weight of the fish did not have a significant effect on the ability of the group to match the distribution of food ($F_{1,6}=0.686$, $p=0.439$).

The stickleback showed large deviations from the predictions of the ideal free distribution in all three of the travel conditions (Figure 2-4). The fish undermatched the proportion of food that was available in all cases, meaning more fish fed at the poor patch, and fewer fish fed at the rich patch. Both the covariate travel cost ($F_{2,46}=3.63$, $p=0.034$) and the food distribution ($F_{1,46}=11.46$, $p=0.001$) significantly affected the distribution of the fish.

The distribution of fish most closely matched the predictions of the IFD in the medium travel cost treatment (slope = 0.404 ± 0.054, $t(2)=-10.994$, $p=0.008$; intercept = 36.723 ± 9.906, $t(2)=3.707$, $p=0.066$), when 6 tunnels were available. Three of the points

fall most closely to the IFD line for the medium group, with two of measurements falling almost directly on the predicted x=y line (Figure 2-4). The low cost regression line better matches the IFD prediction regression (slope = 0.444 ± 0.056, t(2)=-10.116, p=0.010; intercept = 48.887 ± 13.114, t(2)=3.728, p=0.065) than does the high cost regression line (slope = 0.285 ± 0.066, t(2)=-10.873, p=0.001; intercept = 60.469 ± 12.492, t(2)=4.841, p=0.016). However, in three of the 5 food level treatments, the average proportion of fish feeding in chamber A was closer to the predictions of the IFD in the high travel cost group than the low travel cost group (Figure 2-4).

The two most extreme food distributions (1:9 distribution and 9:1 distribution) elicited the largest changes in fish distribution over the course of the trials. In the treatment where 10% of the food was provided in chamber A, there was more of a movement of fish towards the higher output feeder in the low and medium travel cost treatment than the high cost treatment (Figure 2-5). There was virtually no change in the proportion of fish feeding throughout the trial in the high travel cost treatment (3 tunnels available). Alternatively, when 90% of the food was provided in chamber A, the groups in all three travel cost treatments showed an increase in the proportion of fish feeding at the higher-output feeder over the course of the 25-minute trial (**Error! Reference source not ound.**). There was no significant change to the proportion of fish feeding in chamber A in the less extreme and equal food conditions over the course of the trials, regardless of travel costs (**Error! Reference source not found.** -

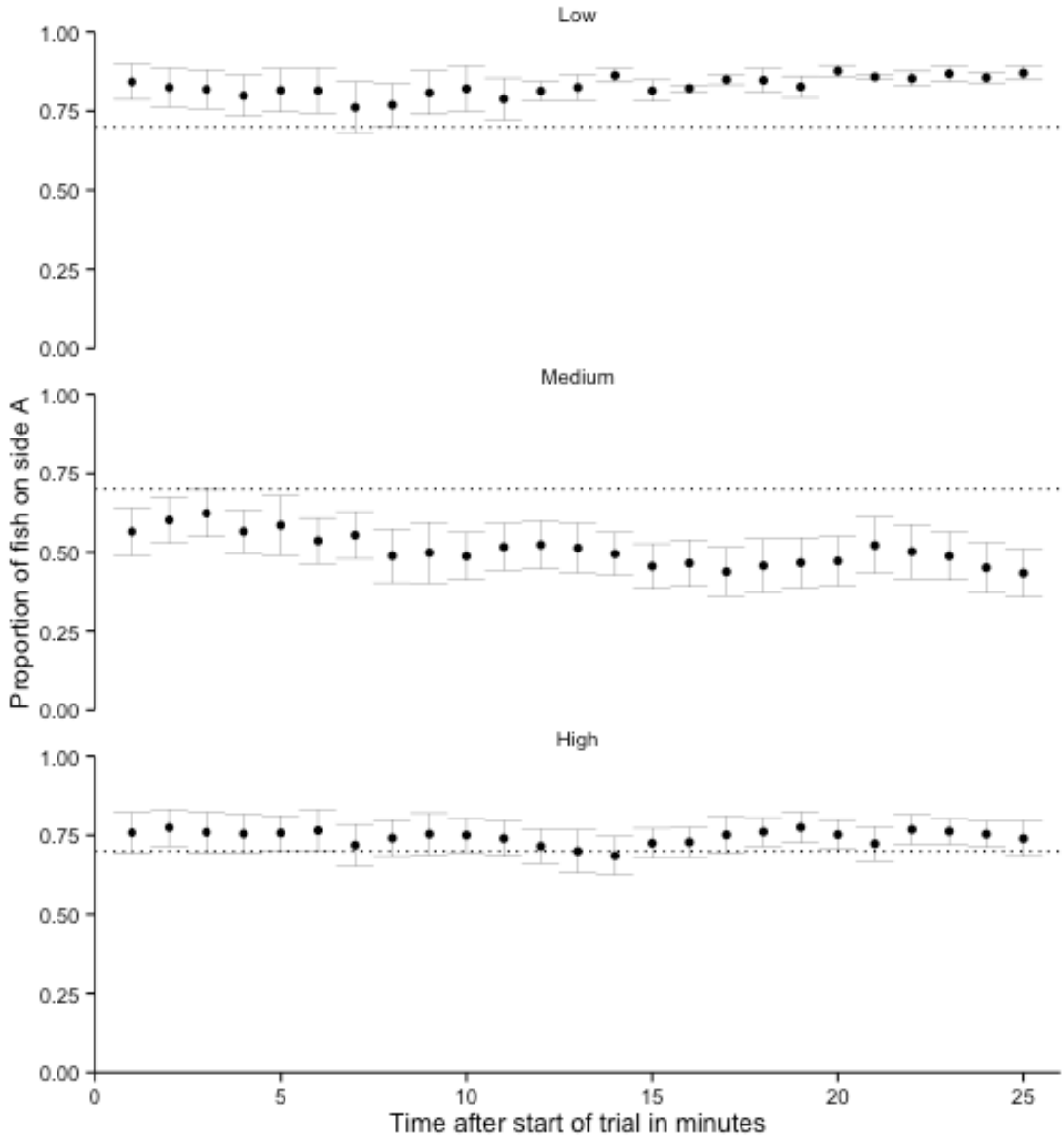

Figure 2-9 The proportion of stickleback between two continuous input food patches with a profitability ratio of 7:3 over the course of 25-minute feeding trials at different travel cost levels. Travel costs treatment was achieved by manipulating the number of tunnels available to the fish to swim between feeding chambers. Low cost treatment had all nine tunnels available; medium cost had six tunnels open; and high cost had three tunnels

open. Groups of fish (n=10) were assigned to a travel cost level for all feeding trials. The mean number of fish (means of 3 trials with 3 groups for the low and medium travel cost treatment, and 3 trials with 4 groups for the high travel cost treatment) in the more profitable patch is shown by filled circles; bars show standard error; dotted lines show the predicted number of fish based on patch profitability.

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2.5 Discussion

When animals travel between food patches, they pay a cost to move from one location to the other. This cost can be divided into two distinct portions: the physical cost of movement (energy), and the opportunity cost of movement (missed feeding opportunities incurred by increased time between patches). With the experimental design described here, I was able to not only decouple the two costs, but also remove other extraneous variables – particularly the confounding perception limitations that come along with increased distance between food patches (Roberts and Goldstone 2005). This allowed me to study solely the impact of increased travel time on the ability of stickleback to match a resource distribution between two food patches. I hypothesized that, as the time needed to travel between patches increased, fewer sticklebacks would move between the patches, and therefore their ability to match the resource distribution would be reduced.

The first experiment successfully demonstrated that manipulating the number of tunnels available for travel using clear barriers was a useful way to increase the difficulty of travel between two food patches. Two measurements – switch rate between patches

and change in distribution – both revealed that travel became more difficult for the stickleback when fewer tunnels were available. When a single tunnel was open for travel, representing 0.98% of the potentially available area, most of the fish remained at their starting food patch, and did not sample the second food patch. Additionally, in some trials, the busier food patch attracted fish from the chamber that contained a smaller starting population, which caused the distribution to become even more extreme. This matches previous results which have shown that when they are unable to obtain patch quality information through sampling, stickleback will forage with a larger shoal (Milinski 1979, Gotceitas and Colgan 1991, Krause 1992, Webster and Hart 2006).

The results of the second experiment showed conformity to the hypothesis, where there were trends towards less movement and greater discrepancies from the distribution predicted by the IFD as the cost of travel increased. However, the relationship appears to be more complicated than was expected, as the best fit to the IFD was seen at a medium travel cost, where 6 tunnels were available for travel between the feeders. Groups of stickleback that could most easily move between food patches (all tunnels were open) were only slightly closer to the predicted distribution than those where travel was the most difficult. While the overall distributions were similar between the groups subjected to the highest and lowest travel cost treatments (Figure 2-4), their movement throughout the trials was quite different. The time series graphs (Figure 2-5 to **Error! Reference source not found.**) show that the highest travel cost groups had the greatest amount of inertia in movement: their distribution remained stable throughout the whole trial, meaning there was little or no travel between the two food patches. In the low cost trials, there was greater movement towards an IFD over the course of the 25-minute trial,

despite the fact that this distribution was never achieved. This was particularly visible at the most extreme food distributions: 10% and 90%.

In experiment 1, the switching rate was much lower as travel became more difficult. This result was also seen in a simulation experiment using a learning rule (Regelmann 1984) and in pigeons (Baum and Kraft 1998). Additionally, while it was not measured directly, Kennedy and Gray (1997) observed that more ducks switched between food patches that were separated by 16 m than when the patches were 45 m apart. While I did not measure switching in my second experiment, I noted that there was less variation in the fish's distribution across trials when travel costs were high (Figure 2-5 to **Error! Reference source not found.**), indicating that there were fewer fish straying from the feeder at which they began.

The experiments were designed to control for possible confounding factors that are known to cause overmatching. These include changes to the absolute resource rate, population size, and food dispersal area (Roberts and Goldstone 2005). The resource input was maintained at 100 food units (individual brine shrimp) per 25-minute feeding trial, which were delivered within the same size area by an automated feeder. This method controlled both the absolute resource rate, and the food dispersal area. The number of individuals within the experimental groups was always 10 and any trials with a mean participation rate of less than 60% were removed from analysis, so competition for food was similar for all trials.

The results of these two experiments are similar to the findings of several other studies on the effects of travel cost, in which increasing the cost to travel between resource patches tended to cause undermatching. Travel costs have been manipulated

experimentally by increasing distance between patches (Kennedy and Gray 1997), placing barriers between patches (Baum 1982), adding a time penalty for "switchers" (Baum 1982, Regelmann 1984), or increasing distance and obstacle level simultaneously (Korona 1990). Two studies have reported overmatching as travel became more costly (Baum 1982, Aparicio 2001). Both studies, Baum's with pigeons and Aparicio's with rats, altered travel costs by increasing the height of a hurdle separating two food patches. However, in Baum's experiment, when only an opaque partition of variable width was used to separate the patches rather than the hurdle, the pigeons undermatched the resource distribution as in the majority of other studies. Theoretically, both overmatching and undermatching have been proposed as the result of a cost of movement (Åström 1994). In his model, Åström demonstrated that the direction of distribution mismatching depends on which direction of movement is more costly. When more energy is required to move from the richer habitat to the poorer habitat, the subjects will have a more extreme distribution than the resource distribution (undermatching). This fits the circumstances of this experiment, as more feeding opportunities are lost by the fish moving from the rich patch to the poor patch.

The inertia seen in the distribution between food patches has several potential causes, outside of the travel costs. Firstly, the overarching assumption of the experimental design was that the stickleback would prioritize energetic gains over any other environmental factor. However, resource acquisition may not have been their main priority. In both experiments, 10 fish were fed approximately 100 individual brine shrimp three times daily. This diet was the estimated energetic requirement to maintain the fish's base body mass. It is conceivable that this feeding level was too high, so the stickleback

did not need to distribute perfectly with the resources to obtain enough energy. The ideal free distribution is only applicable in an energetically stressful environment; in an environment that is rich overall, the animals will typically all congregate in the patch with the highest resource density (Milinski 1994, Tregenza 1994). Even if the diet during the experiment was low enough to be energetically challenging, they may have had enough of an energetic reserve to deal with the shortage during the five-day trial.

Habitat selection decisions are complicated, and can incorporate many different factors. With social species like stickleback, habitat selection can be a function of population density and distribution, where "safety in numbers" is prioritized over resource gains. In the first experiment described above, the population distribution was controlled, where the fish were placed in an 8:2 starting distribution for each trial. While there was either no movement or movement towards the lower populated chamber in most experiments, a few trials showed greater movement toward the chamber with more competition. There was no manipulation of the starting distribution of fish in experiment 2. As can be seen in the time series graphs (Figure 2-5 to **Error! Reference source not found.**), the groups in the medium travel cost treatment were most likely to start out in a more equal distribution. The groups in the low cost and high cost treatments began in a more extreme, unequal distribution, which translated into a greater deviation from the expected distribution throughout the trial.

2.6 Conclusion

With increasing travel costs the rate at which stickleback switched between patches was significantly reduced. Consequently, the distribution of fish increasingly deviated from

the distribution of their food. From the results of these two experiments, it is unclear whether the fish were unable to determine which patch was more profitable when travel was too difficult, or if they simply did not make their habitat selections based on energetic availability. When the travel costs were high, the fish appeared to select their food patch based on the population distribution rather than the resource distribution, causing them to undermatch the expected Ideal Free Distribution. I saw that, overall, when the travel costs were the lowest, the fish showed higher switch rates between patches, and were better able to match the food distribution, but there was an unknown mechanism that caused the groups in the medium travel cost treatment to best match the resource distribution in the second experiment.

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2.8 Tables and Figures

Table 2-1 Mean (± SD) total length (mm) and wet weight (g) for each group (n=10) of threespine stickleback used in experiment 1.

Group	Length	Weight
1	58.49 ± 3.25	1.28 ± 0.23
2	47.18 ± 3.40	0.66 ± 0.14
3	41.30 ± 2.16	0.41 ± 0.08
4	62.09 ± 4.03	1.55 ± 0.30
5	49.17 ± 2.56	0.86 ± 0.13
6	43.05 ± 1.55	0.59 ± 0.09

Table 2-2 Mean (± SD) total length (mm) and wet weight (g) for each group (n=10) of threespine stickleback used in experiment 2.

Group	Length	Weight
1	41.44 ± 1.97	0.60 ± 0.09
2	49.45 ± 2.45	1.06 ± 0.17
3	43.33 ± 1.93	0.65 ± 0.11
4	44.69 ± 0.77	0.75 ± 0.09
5	51.08 ± 1.38	1.18 ± 0.19
6	41.32 ± 1.39	0.55 ± 0.04
7	46.79 ± 1.79	0.84 ± 0.09
8	51.45 ± 2.18	1.13 ± 0.15
9	45.14 ± 2.24	0.73 ± 0.09
10	39.67 ± 2.21	0.50 ± 0.06
11	45.45 ± 2.28	0.74 ± 0.06
12	44.69 ± 1.06	0.73 ± 0.07

Table 2-3 Participation (mean ± SD) for the six groups of fish (n=10) used in Experiment 1. Overall participation was calculated from the mean participation of all 15 trials. Travel cost-specific participation was calculated as the mean over the five trials of each travel cost treatment.

Group	Overall participation	Participation in high cost trials	Participation in medium cost trials	Participation in low cost trials
1	7.85 ± 0.25	8.00 ± 0.24	7.89 ± 0.22	7.67 ± 0.21
2	8.79 ± 0.21	8.87 ± 0.30	8.72 ± 0.18	8.78 ± 0.13
3	6.96 ± 0.67	7.20 ± 0.67	6.98 ± 0.79	6.68 ± 0.59
4	8.04 ± 0.62	8.00 ± 0.53	8.32 ± 0.36	7.63 ± 1.02
5	7.34 ± 0.66	7.27 ± 0.74	7.49 ± 0.61	7.27 ± 0.73
6	8.49 ± 0.53	8.27 ± 0.49	8.71 ± 0.62	8.50 ± 0.49

Table 2-4 Mean number of switches (± SE) between two equal food patches by 6 groups (n=10) of threespine stickleback over 25-minute feeding trial when travel cost was manipulated. Travel costs treatment was achieved by altering the number of tunnels available to the fish to swim between feeding chambers. Low cost treatment had all five tunnels available; medium cost had three tunnels open; and high cost had a single tunnel open. Five trials were completed at each cost level for each group. Three outlier trials were removed prior to calculating the mean and SE. Post-hoc analysis demonstrated that the High cost travel treatment was significantly different (*) from both the Medium cost and Low cost treatments.

Group	High costs*	Medium costs	Low costs	Σ total crossings
1	0.75 ± 0.25	8.00 ± 1.45	26.60 ± 7.34	183
2	0.40 ± 0.25	2.00 ± 0.71	3.80 ± 2.20	50
3	0.40 ± 0.25	3.60 ± 1.21	5.60 ± 2.46	48
4	0.60 ± 0.40	6.20 ± 2.85	24.40 ± 2.91	156
5	1.25 ± 0.48	20.80 ± 5.62	32.80 ± 6.80	285
6	1.20 ± 0.37	7.00 ± 1.14	12.60 ± 1.63	104

Table 2-5 Participation (mean ± SD) for the 12 groups of fish (n=10) used in Experiment 2. Mean participation was calculated by taking the average participation across the 3 trials of each food distribution treatment. These five values were then used to calculate the overall mean and the standard deviation.

Group	Travel Treatment	Participation
1	Low cost	2.35 ± 0.47
2	Medium cost	3.35 ± 0.90
3	High cost	8.03 ± 0.19
4	Low cost	8.26 ± 0.76
5	High cost	7.35 ± 0.60
6	Medium cost	6.00 ± 0.99
7	Low cost	8.65 ± 0.37
8	High cost	8.81 ± 0.32
9	Medium cost	8.37 ± 0.68
10	Medium cost	8.20 ± 0.56
11	High cost	8.02 ± 0.30
12	Low cost	6.18 ± 0.55

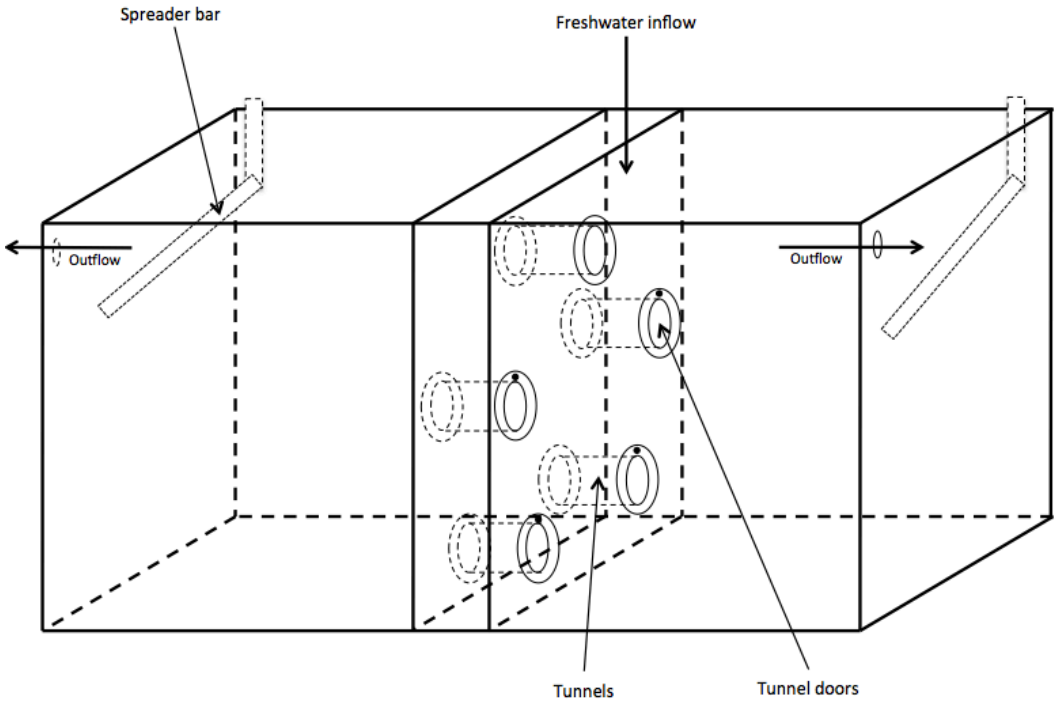

Figure 2-1 Schematic diagram of the apparatus used for experiment 1 (not to scale). The apparatus was created by installing two clear plexiglass partitions into a 91 cm (L) x 46 cm (W) x 36 cm (H) glass aquarium. Food entered the apparatus through the two spreader bars, located at either end of the aquarium. This divided the tank into two 41.5 cm end chambers, divided by an 8 cm centre compartment. Five tunnels (5.08 cm inner diameter, 8 cm in length) arranged in a pentagonal formation joined the two compartments together across the centre[1]. Each tunnel had a clear plexiglass door at either end to close off access to the tunnel. In the first experiment, the doors rotated on a single screw, allowing the tunnels to be covered and uncovered with minimal disturbance to the test animals. Two outflow holes, one at either end of the tank, were drilled 30 cm from the bottom.

[1] A similar aquarium setup was used for the second experiment, with a few important differences: the chambers were joined by 9 tunnels, arranged in a 3x3 grid; and, the clear vinyl doors at either end of the tunnels were not hinged. They were screwed into place with two stainless-steel screws before the 5-day experiments began.

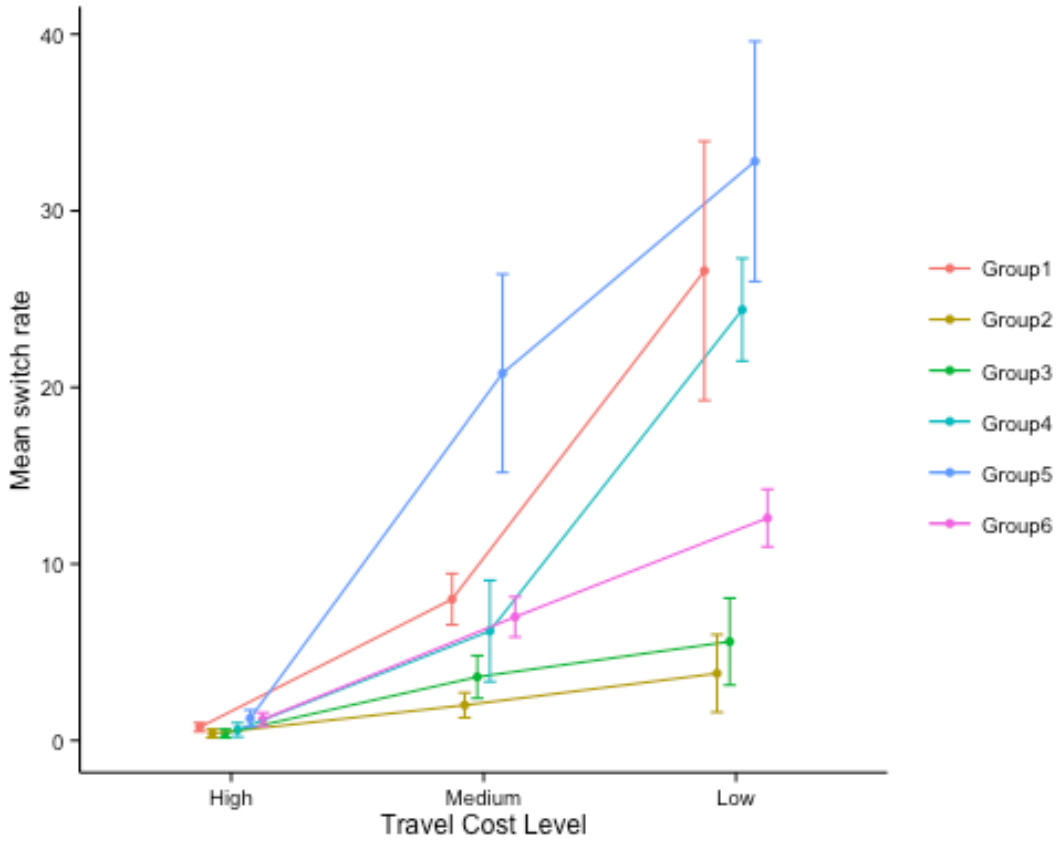

Figure 2-2 Line graph of the mean (±SE) number of times a fish travelled from one chamber to the other (switch rate per 25 minute trial) that occurred in six groups of 10 fish when travel costs to move between two food patches were manipulated (High travel cost = one tunnel available for travel, Medium travel cost = three tunnels available, Low travel costs = five tunnels available).

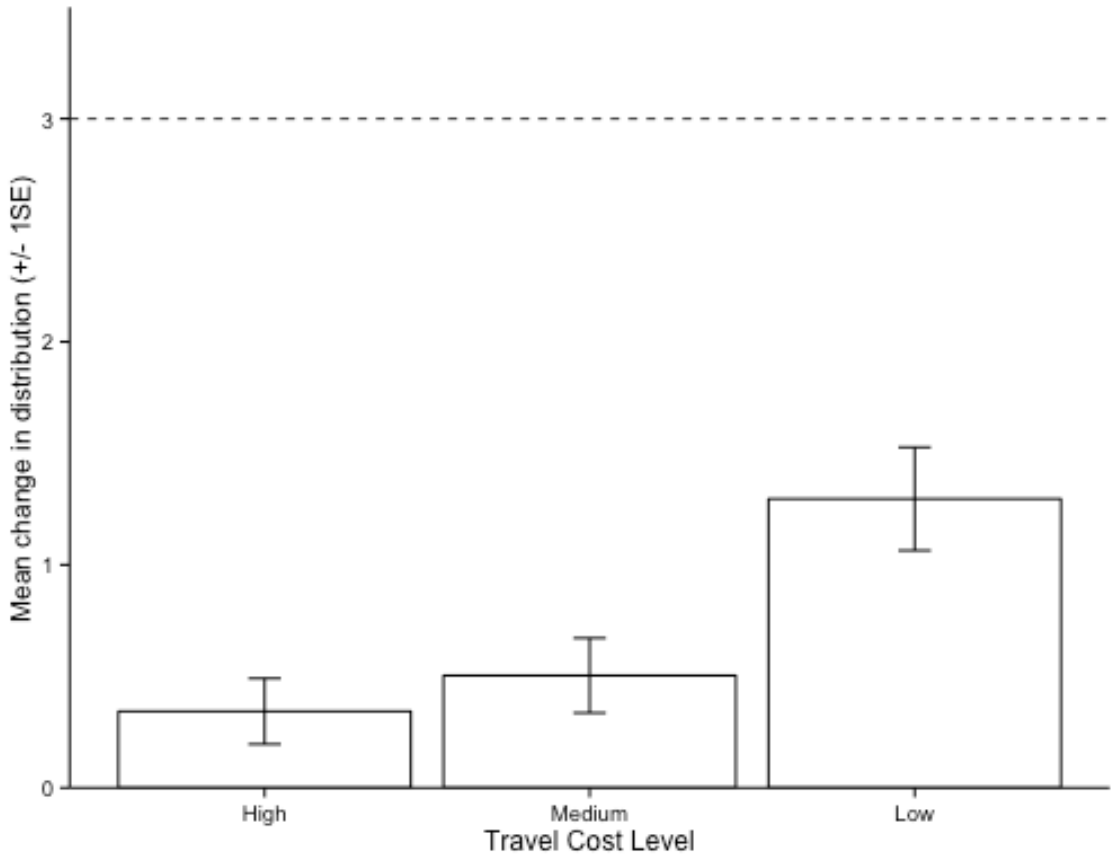

Figure 2-3 The average change in distribution (± SE) of fish that moved from a more populated feeder (8 fish) to a feeder with fewer competitors (2 fish) with equal food delivery. The hashed line represents the change in distribution needed to achieve a distribution of fish that would be equal to the food distribution.

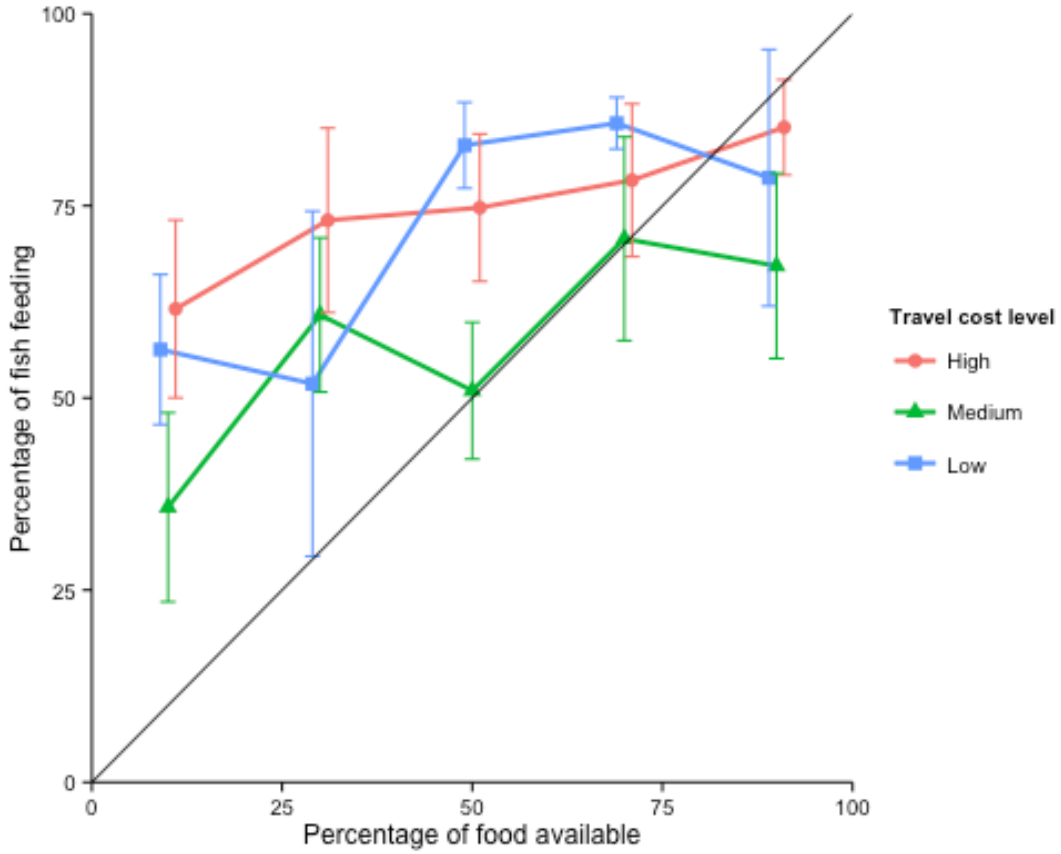

Figure 2-4 Line graph of the average percentage of fish feeding in chamber "A" (±SE) as a function of percentage of food available in that patch. The solid black line represents the expected ideal free distribution (x=y). X values (percentage of food) have been offset by ±1 to better visualize error bars. Travel costs were altered by providing more tunnels for travel between food patches; where high travel cost = three tunnel available, medium travel cost = six tunnels available, and low travel costs = nine tunnels available.

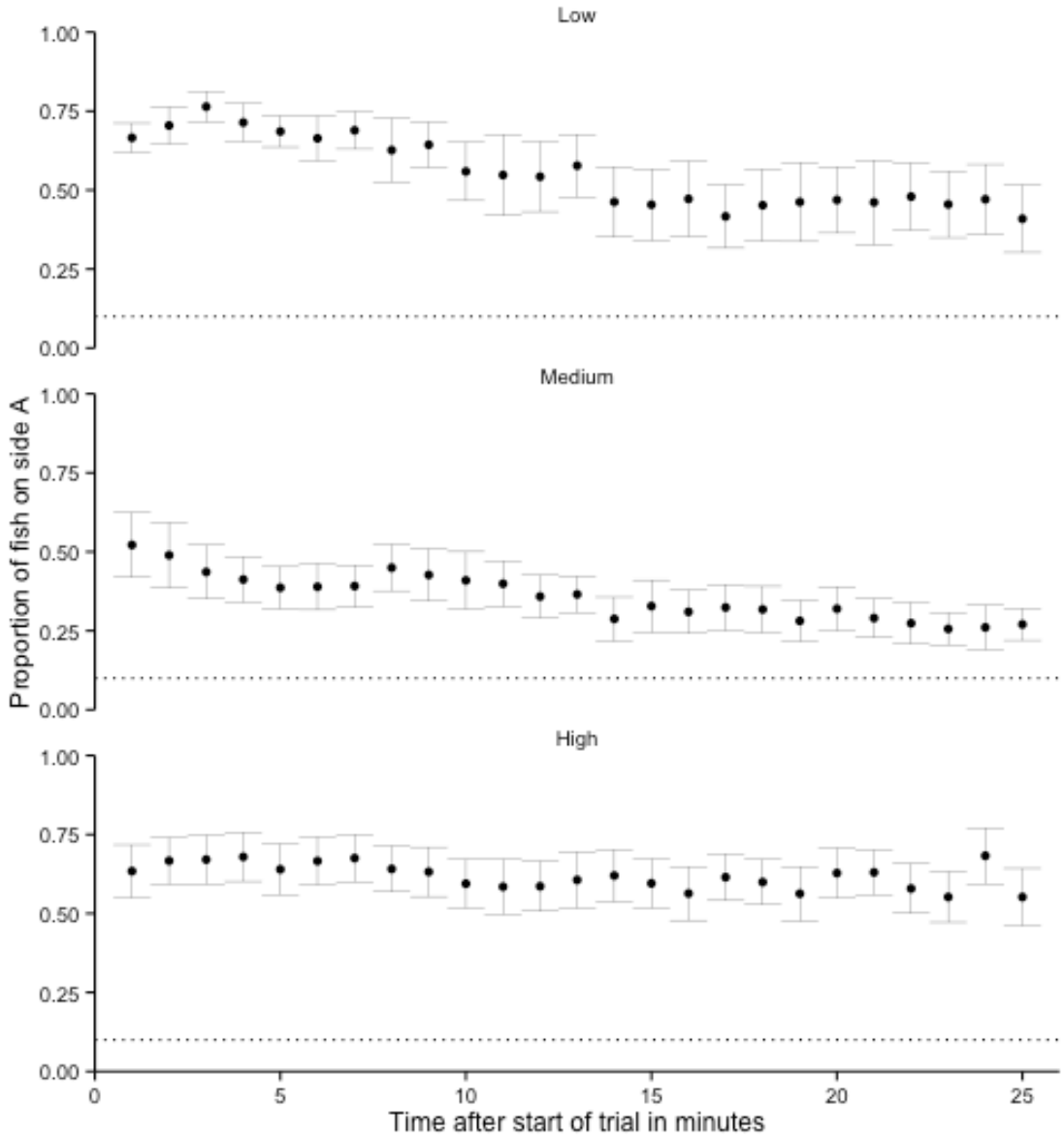

Figure 2-5 The proportion of stickleback between two continuous input food patches with a profitability ratio of 1:9 over the course of 25-minute feeding trials at different travel cost levels. Travel costs treatment was achieved by manipulating the number of tunnels available to the fish to swim between feeding chambers. Low cost treatment had all nine tunnels available; medium cost had six tunnels open; and high cost had three tunnels open. Groups of fish (n=10) were assigned to a travel cost level for all feeding trials. The

mean number of fish in the less profitable patch (means of the 3 trials with 3 groups for the low and medium travel cost treatment, and 3 trials with 4 groups for the high travel cost treatment) is shown by filled circles; bars show standard error; dotted lines show the predicted number of fish based on patch profitability.

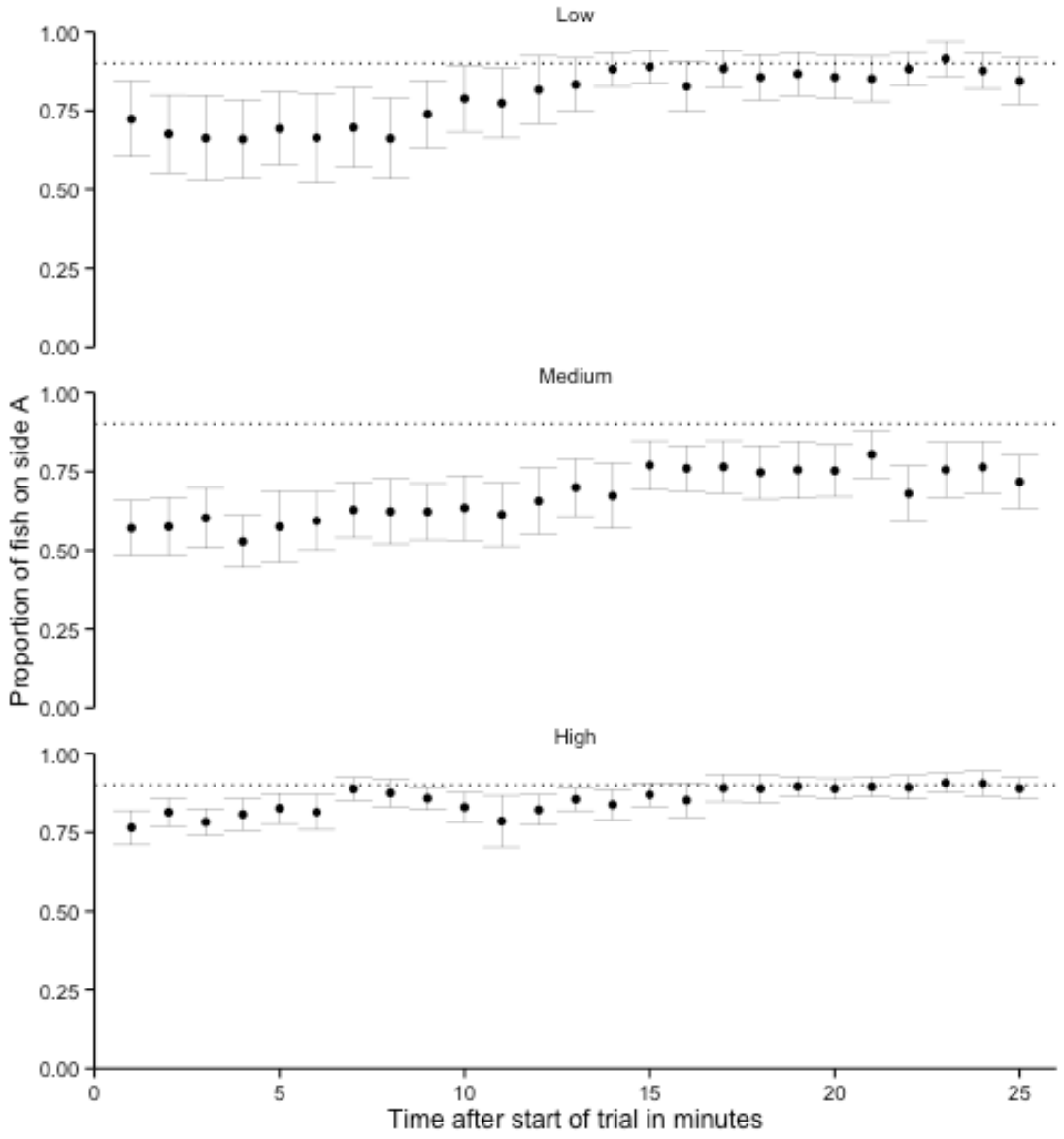

Figure 2-6 The proportion of stickleback between two continuous input food patches with a profitability ratio of 9:1 over the course of 25-minute feeding trials at different travel cost levels. Travel costs treatment was achieved by manipulating the number of tunnels available to the fish to swim between feeding chambers. Low cost treatment had all nine tunnels available; medium cost had six tunnels open; and high cost had three tunnels

open. Groups of fish (n=10) were assigned to a travel cost level for all feeding trials. The mean number of fish (means of 3 trials with 3 groups for the low and medium travel cost treatment, and 3 trials with 4 groups for the high travel cost treatment) in the more profitable patch is shown by filled circles; bars show standard error; dotted lines show the predicted number of fish based on patch profitability.

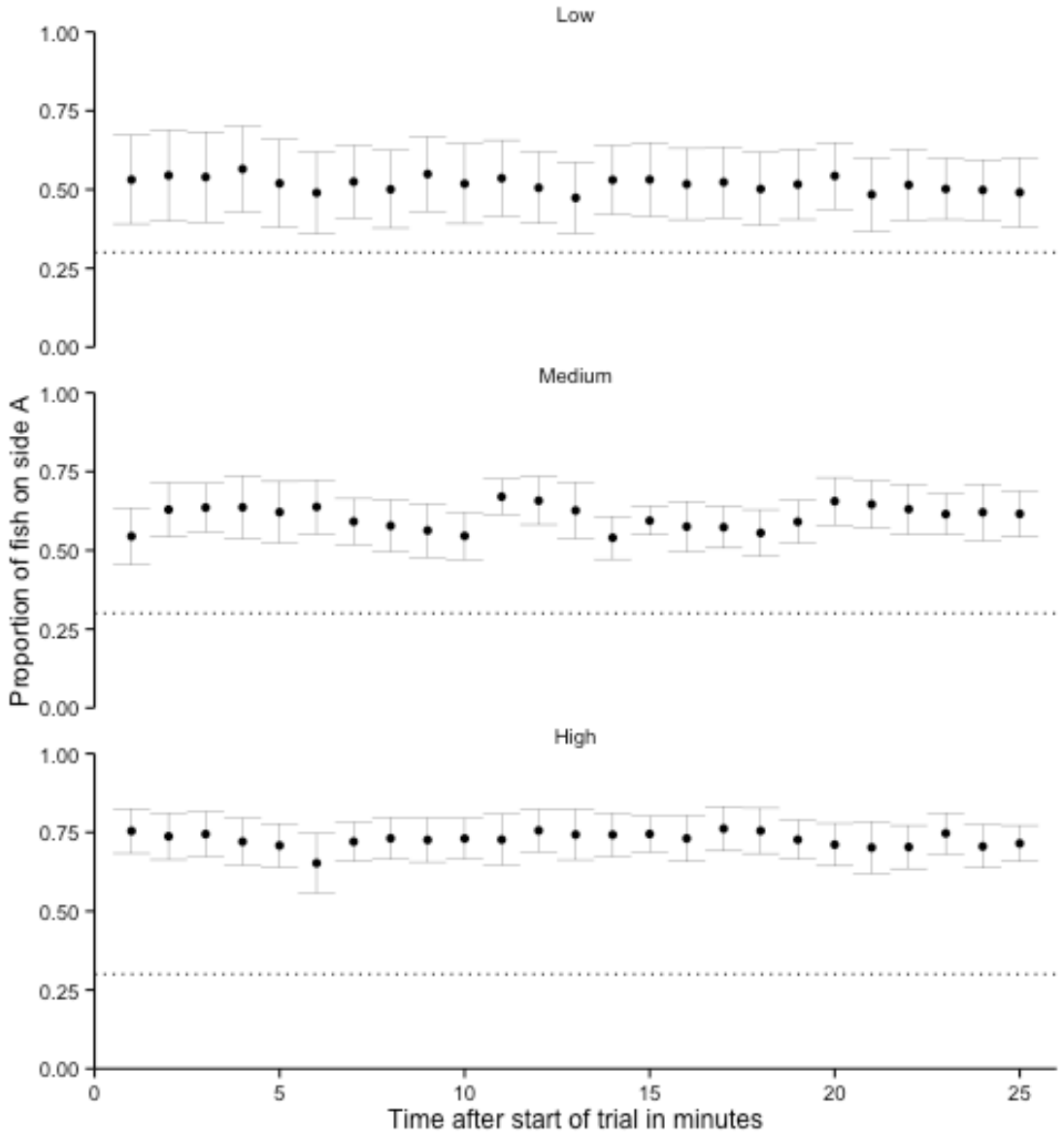

Figure 2-7 The proportion of stickleback between two continuous input food patches with a profitability ratio of 3:7 over the course of 25-minute feeding trials at different travel cost levels. Travel costs treatment was achieved by manipulating the number of tunnels available to the fish to swim between feeding chambers. Low cost treatment had all nine tunnels available; medium cost had six tunnels open; and high cost had three tunnels

open. Groups of fish (n=10) were assigned to a travel cost level for all feeding trials. The mean number of fish (means of 3 trials with 3 groups for the low and medium travel cost treatment, and 3 trials with 4 groups for the high travel cost treatment) in the less profitable patch is shown by filled circles; bars show standard error; dotted lines show the predicted number of fish based on patch profitability.

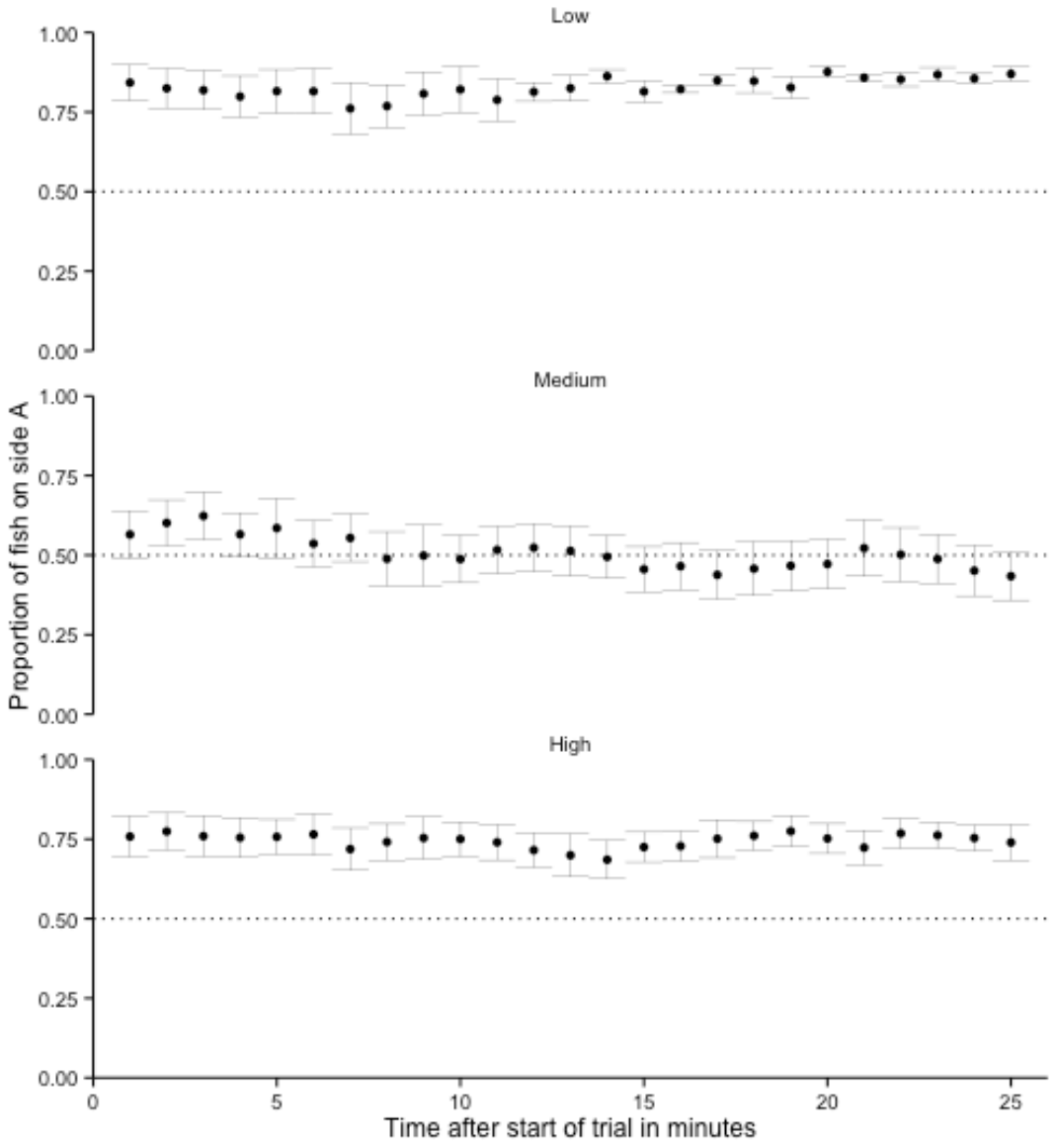

Figure 2-8 The proportion of stickleback between two continuous input food patches with a profitability ratio of 5:5 over the course of 25-minute feeding trials at different travel cost levels. Travel costs treatment was achieved by manipulating the number of tunnels available to the fish to swim between feeding chambers. Low cost treatment had all nine tunnels available; medium cost had six tunnels open; and high cost had three tunnels

open. Groups of fish (n=10) were assigned to a travel cost level for all feeding trials. The mean number of fish (means of 3 trials with 3 groups for the low and medium travel cost treatment, and 3 trials with 4 groups for the high travel cost treatment) in the patch is shown by filled circles; bars show standard error; dotted lines show the predicted number of fish based on patch profitability.

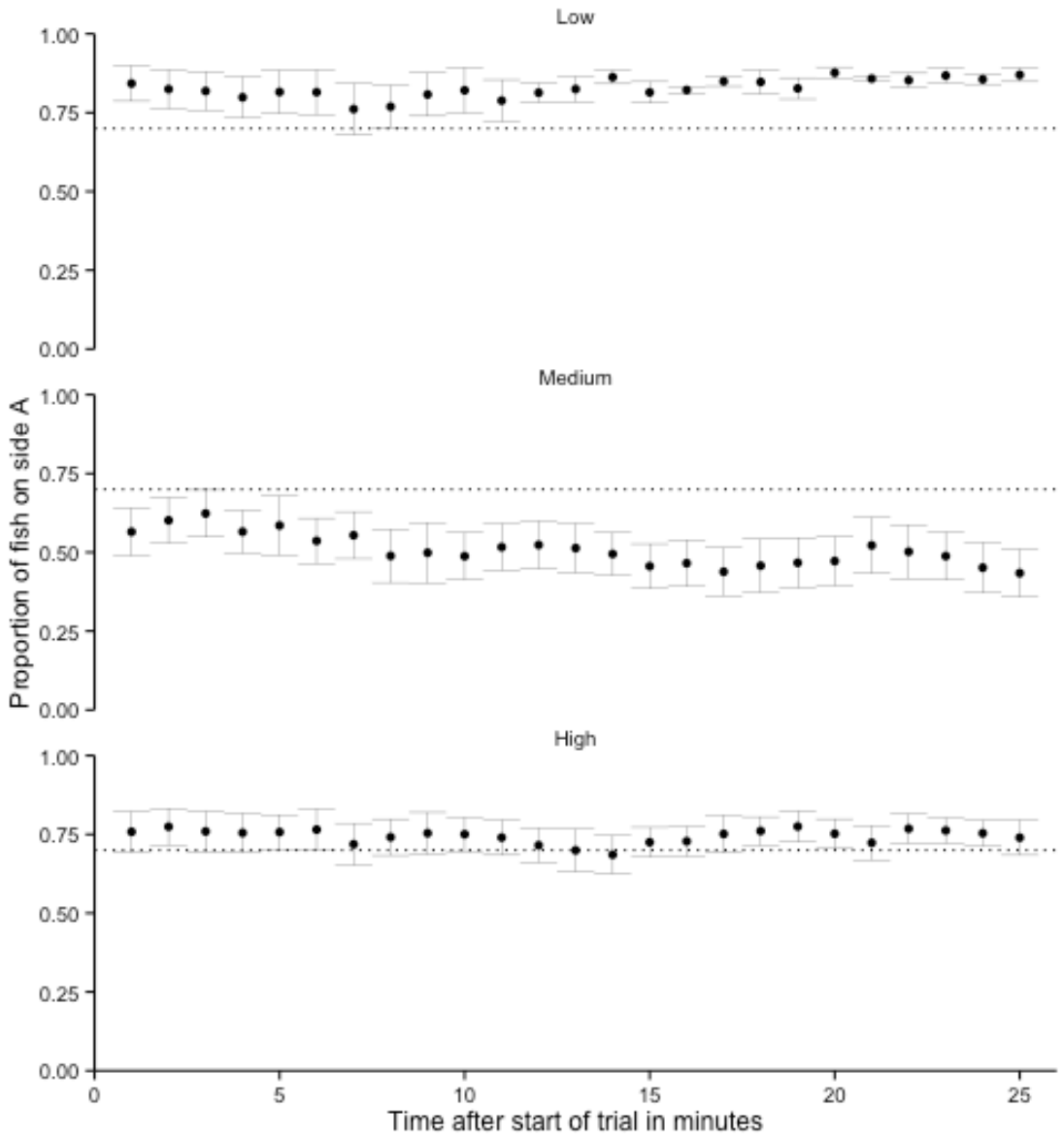

Figure 2-9 The proportion of stickleback between two continuous input food patches with a profitability ratio of 7:3 over the course of 25-minute feeding trials at different travel cost levels. Travel costs treatment was achieved by manipulating the number of tunnels available to the fish to swim between feeding chambers. Low cost treatment had all nine tunnels available; medium cost had six tunnels open; and high cost had three tunnels

open. Groups of fish (n=10) were assigned to a travel cost level for all feeding trials. The mean number of fish (means of 3 trials with 3 groups for the low and medium travel cost treatment, and 3 trials with 4 groups for the high travel cost treatment) in the more profitable patch is shown by filled circles; bars show standard error; dotted lines show the predicted number of fish based on patch profitability.

Chapter 3
Will stickleback use turbid environments to avoid predator detection?

3.1 Abstract

Turbid habitats have been shown to be beneficial locations for many small planktivorous fish and juveniles of some species. Veiling by small suspended sediments provide smaller fish with a visual cover, reducing detection rates by predators and thus alleviating some of the risk of being killed. In this study, I used a two-factor design to investigate whether threespine stickleback *Gasterosteus auleatus* would choose to feed in turbid habitats in the presence of a brook trout *Salvelinus fontinalis* predator. My results were dissimilar from previous studies. While the threespine stickleback did avoid feeding in risky patches, as I expected, they showed no preference for turbid food patches. Even when the clear and turbid food patches were both risk-free, fewer fish fed within the turbid patch. I hypothesize that native environment has a large impact on whether fish are able to use turbid environments for predator avoidance when they are made available. This may be due to physiological adaptation, behavioural adaptation, or a combination of the two.

3.2 Introduction

Lifetime fitness of an animal relies upon its choice of habitat, where habitat suitability is determined by specific physical and biological characteristics of the environment (Hall et al. 1997). Habitats, which are considered to be any area that provides the necessary conditions for an organism's survival, can vary spatially and

temporally in their level of suitability (Hall et al. 1997). Animals are able to accurately assess habitat conditions (Lima and Dill 1990) and choose the best one available to suit their current needs (Johnson 1980, Hall et al. 1997, Jones 2001). Large-scale habitat selection is typically constrained by physical parameters (e.g. temperature, salinity, pH) (Blaber and Blaber 1980), while smaller scale habitat decisions typically focus on maximizing resource acquisition.

Two of the most important biological parameters for patch choice are food availability and safety. It has been well documented that these two factors are often the subject of a risk-tradeoff balance (Abrahams and Dill 1989, Lima and Dill 1990), wherein animals will forgo areas with high food abundance for areas that are safer. Animals can achieve their need for safety by either relocating to areas with fewer predators, or by using cover to avoid detection or to increase chance of escape if they are detected (Abrahams 2005). Cover often refers to vegetation or other sources of habitat complexity (Gregory and Levings 1996, Giannico and Healey 1999, Allouche 2002), but in aquatic habitats, some fish are able to find cover in areas of increased turbidity.

The turbidity as cover hypothesis purports that areas with moderate to high suspended sediment loads provide a visual refuge from predation risk (Gregory 1993, Aksnes and Utne 1997, Chiu and Abrahams 2010). Because of their larger search volumes, piscivorous fish suffer greater impairments to their visual range in turbid waters than do planktivores (Giske et al. 1994, Utne-Palm 2002). The reaction distances of large fishes have been shown both theoretically (Aksnes and Giske 1993, Aksnes and Utne 1997) and experimentally (Crowl 1989, Barrett et al. 1992, Benfield and Minello 1996, Vogel and Beauchamp 1999, Sweka and Hartman 2001, 2003, Harvey and White 2008)

to become smaller as turbidity increases, resulting in a lowered prey encounter rate. In many cases, this results in decreased foraging success (de Robertis et al. 2000, Sweka and Hartman 2001, Gadomski and Parsley 2005, Radke and Gaupisch 2005, Pekcan-Hekim and Lappalainen 2006, Ohata et al. 2011, VanLandeghem et al. 2011, Huenemann et al. 2012), or altered prey selectivity (Abrahams and Kattenfeld 1997). Planktivores can experience improved foraging efficiency in moderately turbid environments (Boehlert and Morgan 1981, Gregory and Northcote 1993, Utne-Palm 1999) because moderate levels of suspended sediments increases the contrast between small plankton and their background (Utne-Palm 2002). Planktivorous fish can therefore benefit from both improved foraging success and lowered risk of predation in turbid habitats.

Human activities have increased turbidity globally, by increasing suspended sediment loads and increasing eutrophication (Tuomainen and Candolin 2011). Anthropogenically induced turbidity can decrease fish population sizes (Moran et al. 2010), reduce species diversity (Hart 1988, Henley et al. 2000, Donohue and Irvine 2004), and cause drastic changes to aquatic community structures (Eiane et al. 1999, Henley et al. 2000, Utne-Palm 2002, Donohue and Molinos 2009, Kemp et al. 2011). In turbid habitats, planktivores dominate the fish assemblage (Jeppesen et al. 1999), and piscivores may be completely absent (Eiane et al. 1999).

Threespine stickleback are a pervasive species of fish, present in freshwater, estuarine, and marine systems across the Northern Hemisphere, and are a species that can play an important role in community structure (Harmon et al. 2009). They are both an important prey item for piscivorous predators (L'Abée-Lund et al. 1992), and a voracious zooplankton predator. Because of their important place within many aquatic communities,

it is important to determine how their habitat choices are affected by turbidity.

Previous work by Chiu and Abrahams (2010) demonstrated that fathead minnows *Pimephales promelas* preferred to feed in turbid patches over clear, regardless of predation risk in this location. This suggested that fathead minnows perceived a significant benefit from the presence of turbidity that outweighed possible harmful effects of the suspended sediments. In Newfoundland, Canada, freshwater fish are not typically exposed to turbidity, as the rocky island has little soil to cause suspended sediment. The fish from this area are therefore naïve to turbidity, unlike the fathead minnows from Manitoba, who live in habitats that are variably turbid (Chiu and Abrahams 2010). It is currently unknown whether the preference for turbid environments by small fish is a learned or innate behaviour. I therefore sought to test whether a population of small planktivorous fish, the threespine stickleback, from a clear water pond in Newfoundland would recognize safety in turbid environments in the same way that fathead minnows do. Using methods similar to those of Chiu and Abrahams (2010), I set out to assess habitat selection by threespine stickleback that were given the choice between two habitats with identical foraging opportunities, but differences in safety and water clarity. I predicted that the threespine stickleback will demonstrate a preference for turbid water when it is safe and when it is risky, despite their inexperience with turbid habitats, as it will provide the same antipredator benefits as it does for other small species of fish.

3.3 Methods

3.3.1 Fish collection and maintenance

Threespine stickleback *Gasterosteus aculeatus* were collected between June and

September 2013 in the same manner as described previously (Chapter 2, p. 9-10). Stickleback were held in 38 L glass aquaria at a density of 50-75 fish per aquaria. Tanks were supplied with an oxygenated flow-through water supply, natural ambient light, and ambient temperature levels, which ranged from 8°C in the winter to 17°C in the summer. The stickleback were fed 1-2 times daily *ad libitum* with TetraMin tropical fish flakes and thawed brine shrimp *Artemia salina*.

Brook trout *Salvelinus fontinalis* were collected from Little Country Pond near Witless Bay, NL (47°17'29.40"W, 52°55'11.20"W) in May 2013 by hook and line. The trout were transported in a large aerated container to the Ocean Sciences Centre, where they were placed in a 300 L flow-through fiberglass conical-bottomed holding tank. The water supply was oxygenated by an Airsep oxygen compressor in a communal header tank that flowed into the trout tank. Oxygen levels were maintained between 100 and 110% saturation and lights were on a 12h:12h light:dark cycle; some ambient light was present from a partially covered window throughout the summer. The water temperature ranged from 18 °C in the summer to 10 °C in the fall. The trout had a mild ectoparasitic infection of freshwater lice (*Argulus* sp., 0-10 lice/fish) that was controlled by manual removal of the lice with tweezers. After collection, the trout were allowed to acclimatize to the laboratory for approximately 2 weeks, then were anesthetized with Eugenol (12µl/L; 1.2mL of 1:9 Eugenol:Ethanol solution in 10L water), measured for wet weight and fork length, and implanted with a 12mm PIT tag. Three trout were used as predators for this study (1: 17.0 cm, 50.0 g; 2: 18.2 cm, 59.1 g; 3: 17.5 cm, 67.1 g). Trout were fed *ad libitum* a diet of fresh live earthworms, dried krill, and 4mm sinking trout pellets. Growth of the trout was minimal over the course of the two-month experiment.

3.3.2 Apparatus

Habitat choice experiments were conducted in an aquarium apparatus (91 cm (L) x 46 cm (W) x 36 cm (H)) where two independent turbidity levels could be generated on each side of a divider while stickleback could maintain free movement between the two 41.5 cm long chambers (Figure 3-1, based on the design in Chiu and Abrahams 2010). The apparatus had a three-chambered system where water flowed into a narrow (8 cm) centre chamber and out towards the two experimental chambers (Figure 3-1). Fish could move between the experimental chambers via five tunnels (5.08 cm inner diameter) that spanned the centre chamber and which were arranged in a pentagonal configuration (see Chapter 2, Figure 2-1). The inflow water flowed from the centre chamber into the end chambers via three 59.5 mm diameter holes that were drilled into each connecting tube. An outflow hole was located at either end of the tank, 30 cm from the bottom. Turbidity was generated by the addition of a bentonite slurry (~2.5g bentonite/L water) to one side of the apparatus by a peristaltic pump. The pressure gradient from the inflow water was high enough to prevent the turbid water from entering the clear chamber of the apparatus. The bentonite diffused through the treatment chamber without mechanical assistance.

The tunnel openings were partially covered using clear acrylic doors to prevent movement of trout between chambers, while permitting free stickleback movement. Three identical apparatuses were constructed in order to run three trials simultaneously.

Two CCTV cameras were used to record activity within each apparatus. The two cameras were placed facing either end of the two chambers in order to obtain the best possible view of fish feeding at either feeder. The two videos were combined onto a single split-screen recording using a Videonix MX-1 video mixer and recorded onto

DVDs for later analysis. This allowed me to see the action at either feeder simultaneously.

3.3.3 Experimental Protocol

The threespine stickleback were trained in groups of 40–50 fish for approximately a week to navigate the apparatus (i.e., to learn to travel freely between the two sides), to recognize a feeder bar as a source of food, and to learn that food was made available at an equal rate to both chambers simultaneously. Training was achieved using an automated feeder placed at either end of the tank that allowed brine shrimp to flow continuously into the two chambers at approximately equal, non-depleting rates for ~30 minutes (see Abrahams 1989 for description). During the training period, two feedings took place each day and the stickleback were provided a daily maintenance diet of brine shrimp at ~10% of their combined body weights. The average weight per fish was estimated from previous experiments, and was multiplied by the number of fish in the group. This collective weight was then used to determine daily maintenance rations required.

Prior to beginning trials, 10 similarly sized (by length) fish were selected from the trained group for each apparatus (average length range: 7.5 mm; maximum range: 16.6 mm). The stickleback were anesthetized using 750 μL of 1:9 eugenol:ethanol solution dissolved in 1 L of water, and measured for total length and wet weight (Table 3-1). The remaining trained fish were kept to replace any mortalities that occurred during trials, and to facilitate training for the subsequent trials. The experiment was completed between July and September 2013 using seven groups of 10 fish.

Each group of fish was subjected to all four experimental conditions for one day

each. Two habitat characteristics, turbidity and predator presence, were combined to create the four different treatment conditions tested in this experiment: (1) clear water and no predator, (2) turbid water and no predator, (3) clear water with a trout predator, and (4) turbid water with a trout predator. Two feeding trials took place each day at 1000 and 1600. Each experiment consisted of one manipulated chamber and one clear (control) chamber, between which the fish could choose where to feed. The manipulation chamber and order of treatments were randomized for each group. For predator treatments, the trout was placed on the appropriate side of the apparatus at 0830, and remained until the conclusion of the second trial of the day. For turbid treatments, the peristaltic pump was turned on an hour before the trial began, which provided enough time to reach the desired turbidity level. The pump was shut off between the two feeding trials of the day.

Equal amounts of food were provided to the two feeders during each feeding trial. Sixty previously frozen brine shrimp were placed in each feeder with 1.5 L of water, allowing the feeders to provide food for 25 minutes. Turbidity (Table 3-2), dissolved oxygen (DO, mg/L) and temperature (°C) were recorded immediately before and after the feeding trials. Turbidity was measured using a Hach Laboratory Turbiditimeter Model 2100D; DO and temperature were both measured using an YSI ProODO. Upon completion of each trial, the number of brine shrimp remaining in the feeders was counted to ensure that they had functioned properly. Feeders were considered to be "equal" if there was a ≤10 discrepancy in the number of shrimp delivered to the two chambers. Trials in which turbidity was not within the acceptable range (<2.5NTU for clear and 10-16 NTU for turbid) or in which the feeders were not equal were excluded and subsequently repeated upon completion of the remaining treatments.

3.3.4 Statistical Analysis

Fish feeding activity was analyzed from the video recordings. The number of fish feeding in each of the two chambers was counted at 30-second increments throughout the trial. The fish were considered to be feeding if they were in close proximity to the spreader bar, were seen to intercept a food item, or darted quickly towards the spreader bar (Chiu 2006). The number of fish seen feeding within ±3 seconds of each 30 s mark was counted for each increment. The mean number of stickleback feeding within each chamber was calculated using only the values acquired after the 3.5 minute mark, following protocols developed by Milinski (1979). This mean was then converted to the proportion of fish feeding in the treatment chamber (calculated as the mean number of fish feeding in treatment chamber divided by the total number of fish feeding). The average of the two feeding trials for each treatment condition for each group was used for analysis.

The fish showed a strong preference for one chamber of the apparatus over the other, regardless of the treatment and treatment location. This bias could have been due to differences in light or background colouration; background pattern and colour is an important element of habitat selection (Kjernsmo and Merilaita 2012). To overcome this side bias, a simple transformation was performed on the proportion values for each group of fish using the following calculation:

$P_{(corrected)} = P_{(observed)} + (0.5 – P_{(CS)})$ for trials in which the manipulation was on the avoided side, and

$P_{(corrected)} = P_{(observed)} - (0.5 – P_{(CS)})$ for trials in which the manipulation was on the preferred side;

in which $P_{(observed)}$ is the original observed proportion of fish in the treatment chamber, $P_{(CS)}$ is the proportion of fish feeding in the treatment chamber during the clear/safe treatment for that group, and 0.5 is the proportion of fish expected to be feeding in the treatment chamber during the clear/safe treatment. The value of $0.5 - P_{(CS)}$ represented the difference between the actual and expected proportion of fish feeding at each chamber when the two patches were identical. In three cases, the transformation caused proportion values to fall outside the possible range of 0-1; two cases where the proportion became negative and 1 where it became greater than 1. In all cases, the value was changed to the closest possible value (i.e., negative values became 0 and values above 1 became 1). Group 4 was removed from analysis due to the presence of a despot, which caused the violation of the IFD assumption that all individuals are free to feed in all patches.

The transformed values were analyzed using a 2-way repeated measured ANOVA, with turbidity and predator presence as the two categorical variables. All analysis was completed using R software version 3.0.

3.4 Results

The six groups of threespine stickleback were found to be significantly different in both length (ANOVA, $F_{5,53} = 11.35$, $p<0.0001$) and wet weight ($F_{5,53} = 17.8$, $p<0.0001$). The groups were divided into three size classes by weight: small (Groups 2 and 7), medium (Groups 1, 5 and 6) and large (Group 3). Size class was therefore included as a between-group factor in the repeated measures ANOVA.

Presence of the predator significantly affected foraging patch choice in threespine stickleback, with the stickleback avoiding feeding in the presence of a predator ($F_{1,3} =$

13.331, p = 0.022; Figure 3-2). Patch use was not significantly affected by water clarity, size, or by the interaction of any factors (size x predation risk, size x water clarity, predation risk x water clarity, and size x predation risk x water clarity) (Table 3-3). Threespine stickleback preferred feeding in the control chamber, which always maintained clear water and safety, to feeding in any of the treatment manipulations (Figure 3-2).

Group participation rates (average number of fish feeding during trials) were similar between the six groups ($F_{5,18}$ = 1.724, p=0.180, Table 3-4), and treatment did not significantly impact participation (ANOVA, Table 3-5). Two participation values (group 3 during turbid feeding when the predator was present (49.1% participation), and group 7 during turbid trials without a predator (68.4% participation)) were low outliers compared to other values from the same treatment, and were therefore excluded from analysis. There was a trend towards lower participation in the presence of the trout predator ($F_{1,5}$=5.334, p=0.069, Figure 3-3). Although the trout were observed to actively track and chase the prey during the feeding trials, no mortalities attributed to the predators occurred during trials.

3.5 Discussion

Many small fish appear to benefit from reduced predator detection in turbid waters, and will therefore preferentially select turbid habitats (Miner and Stein 1996, Gregory and Levings 1998, Chiu and Abrahams 2010). However, it is yet unknown whether fishes native to clear water habitats will utilize turbid water to avoid predator detection. The purpose of this study was therefore to determine how turbidity impacts

habitat choice in threespine stickleback from a non-turbid habitat, and to determine if this species perceives an antipredator benefit to feeding in a turbid environment. I hypothesized that threespine stickleback will be able to take advantage of the turbid water benefits, despite their naivety to this habitat characteristic.

Overall, the data do not support the turbidity as cover hypothesis for this population of stickleback. Our results were consistent with many previous studies in that fewer stickleback fed in the predator-inhabited patches. However, our data diverged from previous studies, as they demonstrated no preference for turbid habitats. Even when the turbid food patch was safe, fewer stickleback fed at this location than was predicted by the food distribution, although water clarity was not a significant factor in analysis. There was significant variation in size among the groups but this did not affect my results. Even though larger individuals tend to be at a reduced risk of predation due to gape limitation of their predators (Biro et al. 2005, Jönsson et al. 2012), this did not impact patterns of habitat selection between the size groups.

Threespine stickleback appear to make different risk-benefit decisions related to turbid waters compared to other studied species. In multiple previous studies, fathead minnows chose to feed in turbid waters above all others, even when the turbid patch was risky and the clear patch was safe (Abrahams and Kattenfeld 1997, Chiu and Abrahams 2010); the stickleback used in my study were relatively impartial to water clarity when it was the only factor. On the other hand, my results are similar to those obtained with marine threespine stickleback from the Baltic Sea, an area that is historically clear but has been increasing in algal turbidity due to anthropogenic eutrophication (Engström-Öst et al. 2009). In this study, the risk level was the same throughout the experimental arena,

and the fish were only given a choice between levels of cover. The marine stickleback demonstrated a preference for clear water over algal turbidity in the absence of a predator signal, but spent equal time in the two habitats when a predator chemical signal was introduced.

Minnows and sticklebacks share similar ecological roles. Species belonging to the two groups are often found together in the same areas (Abrahams 1995, Voellmy et al. 2014), including threespine stickleback and fathead minows (Roberge et al. 2002, Ostlund-Nilsson et al. 2006). Eurasian minnows *Phoxinus phoxinus* and threespine sticklebacks have similar diets (Bolger et al. 1990), as do brook stickleback *Culea inconstans* and fathead minnows (Laurich et al. 2003). Minnows and stickleback are similar sizes, feed on invertebrates and algae, and are also prey to the same piscivorous fishes, birds, and invertebrates.

While turbidity has been found to be the most important physical parameter to influence fish habitat selection (Blaber and Blaber 1980, Cyrus and Blaber 1987a, 1987b, Rodríguez and Lewis Jr. 1997), the mechanism that allows some fish, but not others, to flourish in turbid environments has not yet been elucidated. I suggest that it may be related to adaptation of the sensory systems to the physical characteristics of the native habitat. The results of my study suggest that species and populations that have not been exposed to turbidity in their life history are maladapted to make use of turbid habitats to avoid predator detection when the opportunity is presented.

The effectiveness of a fish's visual system is dependent upon external environmental factors. The ability of a fish to detect a visual target depends on the optical qualities of the object, its background, and the water through which it is seeing (Utne-

Palm 2002), as well as the properties of the eye itself. Physical characteristics of aquatic environments such as ambient light levels, water clarity, and water colour all modify the visual ability of fish (Abrahams 2005). The impacts of turbidity depend on the fish's size and visual field; suspended sediments veil visual targets more for fish with a long visual range, as more particles are present between the eye and the target. For this reason, small fish are able to avoid detection by their larger-bodied predators within turbid habitats. However, predator-prey interactions are not one-sided – whoever detects the other first will often determine the outcome of a predator-prey interaction (Abrahams 2005). While small fish do gain protection from detection within turbid habitats, they may also suffer impairments to detecting the predator, depending upon their primary sensory system for predator detection.

The turbidity as cover hypothesis assumes that both fish are using vision as the primary sense used to detect one another, and that the prey fish are able to detect the predator despite the veiling because it presents as a large visual target. However, my results, in combination with previous studies, indicate that this may be a flawed assumption. Vision is the only sense that is affected by turbidity; fish that use sound and pressure waves or olfaction to detect their predators would be unaffected by turbid water.

The sensory systems of fish are both plastic and adaptable, and can be highly adapted to the physical parameters of their native habitat (van der Sluijs et al. 2011). This is true even among different populations of the same species (McDonald and Hawryshyn 1995). Both specific characteristics and the relative dependence on the different sensory systems can change based on physical environment. For example, the pigments that are expressed and the pattern of rod and cone placement within the retina can be altered over

evolutionary time in order to optimize spectral sensitivity within their native photic regime (Muntz and Mouat 1984, McDonald and Hawryshyn 1995, Horodysky et al. 2010). The relative importance of visual versus olfactory versus pressure cues can also change based on the physical environment (Hartman and Abrahams 2000, van der Sluijs et al. 2011).

There are several ways in which the primary sensory mode, or the way in which sensory compensation takes place, could allow some, but not all, fish to continue to detect their predators in turbid environments. This depends upon the adaptations of the fish to turbid environments. For example, let us look at the fathead minnows used by Chiu and Abrahams (2010). These fish came from an environment where turbidity was a common, but variable, habitat characteristic. Their visual system may have been adapted to function under low light conditions, such as by investing more energy into eye size, as has been seen in Red shiners *Cyprinella lutrensis* (Dugas and Franssen 2012). Another possibility is that when less visual information is available, the fathead minnows alter the importance of different sensory inputs. This alteration is seen in rainbow trout *Oncorhynchus mykiss* (Rowe et al. 2003), which can switch from vision to chemosensory and lateral line input to detect their predators. Fish that reside in environments that do not necessitate complex sensory structures or compensatory mechanisms will not invest energy into these costly systems, as maintaining highly adapted sensory systems is energetically expensive. The stickleback used in this study were collected from a clear-water pond, making it unlikely that they possess adaptations to detect predators in turbid waters, and so tended to avoid turbid habitats.

If we accept the argument that the antipredator benefits of turbid habitats can only

be used by those populations and species that are adapted to do so, there may be wide-reaching implications for water habitat preservation policies. Land-based activities such as agriculture, deforestation, mining, and road construction cause large amounts of sediment to move into the global water systems (Kerr 1995, Wood and Armitage 1997, Donohue and Molinos 2009, Herringshaw et al. 2011). Laws and policies that only allow certain increases in turbidity regulate human-induced changes to water clarity and suspended sediment load. However, most of these policies are currently based on generalized physiological tolerance levels (Kemp et al. 2011) and ignore the possibility of behavioural effects. Based on the results of my study, anthropogenic activities that increase turbidity will impact highly visual species from clear water more than those already residing in variably turbid environments.

3.6 Conclusion

My experiment demonstrated that this population of clear-water threespine stickleback has no preference for turbid environments, regardless of levels of predation risk, unlike fathead minnows (Chiu and Abrahams 2010). The threespine stickleback did not follow the predictions of the turbidity as cover theory, which presumes that they would prefer turbid environments in order to avoid detection by brook trout predators. This dissimilarity is likely due to differences in the physical characteristics within their native habitats. The sensory systems of fish are highly plastic, and adapt to the habitat characteristics in which they reside. I believe that clear water fish, such as the present stickleback, do not use turbid water to avoid their predators because their ability to detect the predator is impaired, and the outcome of predator-prey interactions is often dictated

by who detects whom first. The fathead minnows used by Chiu and Abrahams came from a water system that had large pulses of turbidity that the minnows were adapted to exploit as predator-evasion habitat. I propose that a comparative study of habitat preference between several populations of threespine stickleback, from both natively turbid habitats and clear habitats, would further lend support to this new hypothesis.

3.7 References

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3.8 Tables and Figures

Table 3-1 Average (± SD) total length (mm) and wet weight (g) for each group (n=10) of threespine stickleback used in the experiment.

Group	Length	Weight
1	49.4 ± 2.5	0.84 ± 0.14
2	44.7 ± 1.8	0.60 ± 0.09
3	50.6 ± 2.2	1.19 ± 0.34
4	57.8 ± 5.3	1.56 ± 0.36
5	49.5 ± 2.8	0.73 ± 0.08
6	47.7 ± 1.8	0.81 ± 0.11
7	45.1 ± 2.3	0.55 ± 0.11

Table 3-2 Turbidity values (mean ± SD) of two chambers of a habitat choice aquarium in which turbidity could be manipulated in one chamber (treatment chamber) without impacting the other (control chamber). Measurements were taken in Nephalometric Turbidity Units (NTU) before and after feeding trials with threespine stickleback. Two habitat characteristics, water clarity and predator presence, were combines to create the four treatment conditions. C = clear, T = turbid, P = predator, S = safe (no predator).

Treatment	n	Control chamber (before)	Control chamber (after)	Treatment chamber (before)	Treatment chamber (after)
CS	6	0.73 ± 0.21	0.73 ± 0.29	0.73 ± 0.27	0.75 ± 0.34
CP	6	0.81 ± 0.18	0.81 ± 0.14	1.23 ± 0.80	1.10 ± 0.49
TS	6	1.08 ± 0.42	1.39 ± 0.55	12.89 ± 2.29	12.79 ± 1.99
TP	6	1.21 ± 0.45	1.51 ± 0.66	12.10 ± 1.92	14.10 ± 2.66

Table 3-3 Summary of repeated measures ANOVA on percentages of threespine stickleback feeding in the treatment chamber. Each of the 6 replicates used for analysis had 10 stickleback. Factors: Size (size class, based on weight, of threespine stickleback: large, medium, and small), Water Clarity (clear and turbid), and Predation Risk (absence or presence of a brook trout).

Factors	DF	F	p	Effect size
Size	2	2.383	0.240	0.419
Water Clarity	1	0.386	0.578	0.020
Predation Risk	1	29.427	0.012**	0.685
Size*Water Clarity	2	5.369	0.102	0.358
Size*Predation Risk	2	2.623	0.219	0.279
Water Clarity*Predation Risk	1	0.017	0.903	0.001
Size*Water Clarity*Predation Risk	2	5.734	0.094	0.393

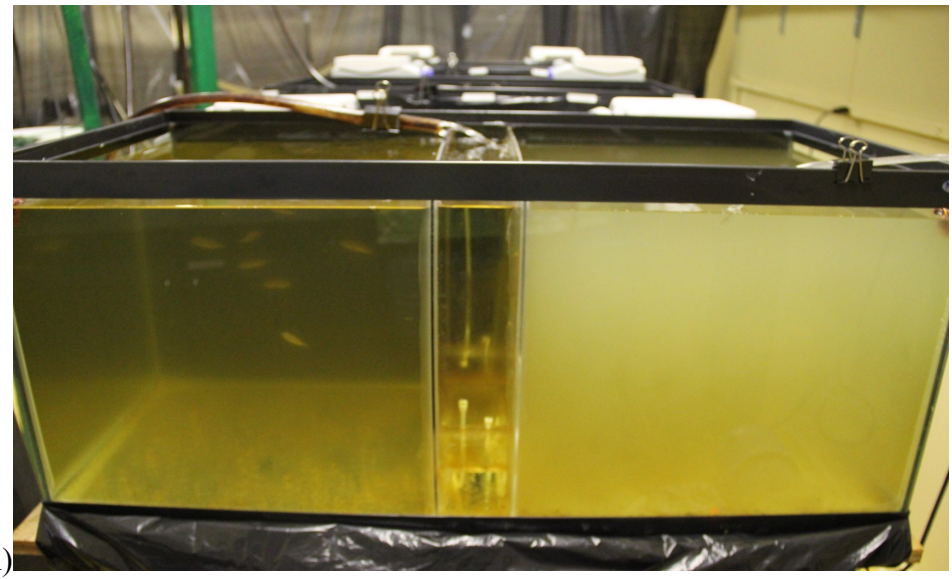
Table 3-4 Fish participation rates (mean ± SD) of the six groups of fish (n=10) used in analysis. Mean participation rate was calculated as the number of fish feeding on average throughout the 25-minute feeding trial, from a total of eight trials with four treatment conditions (2 trials/treatment).

Group	Participation
1	8.43 ± 0.27
2	8.31 ± 0.90
3	6.91 ± 1.44
5	7.98 ± 0.55
6	7.88 ± 0.56
7	7.39 ± 1.02

Table 3-5 Summary of repeated measures ANOVA on participation rates within six groups of 10 threespine stickleback, by treatment. Factors were Water Clarity (clear and turbid), and Predation Risk (absence or presence of a brook trout).

Factors	DF	F	p	Effect size
Water Clarity	1	0.647	0.458	0.017
Predation Risk	1	5.334	0.069	0.180
Water Clarity*Predation Risk	1	0.090	0.777	0.005

A)



B)

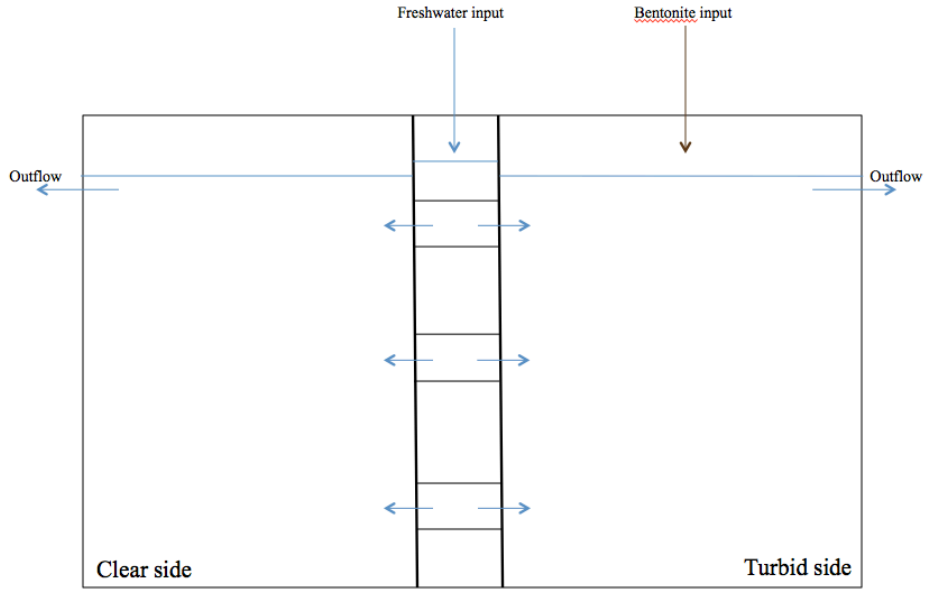


Figure 3-1 A) Photograph of the apparatus at work, illustrating the turbidity gradient between the two chambers of the apparatus (black plastic covering has been removed from the sides to visualize the apparatus for this picture). B) A schematic diagram showing the water flow within the apparatus. Positive pressure in the centre chamber was generated by the freshwater input. This prevented the turbid water from mixing throughout the apparatus. A turbidity gradient of 0 NTU (<2.5 NTU was accepted) in the

clear chamber and 12 NTU (10-16 NTU was accepted range) in the turbid chamber was generated for these experiments.

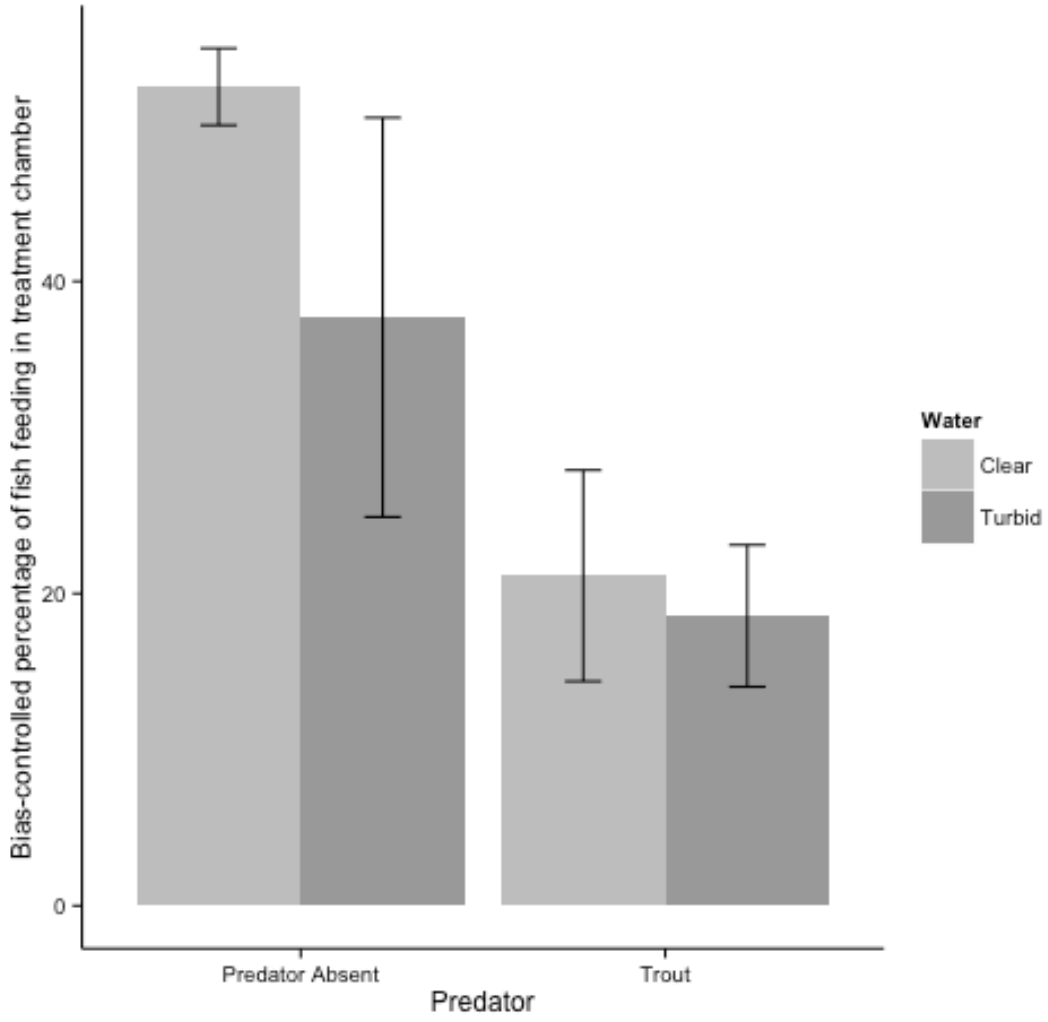


Figure 3-2 Mean percentages of threespine stickleback feeding in the treatment chamber (n = 6 groups of fish). Each group contained 10 stickleback. Error bars represent ± 1 standard error.

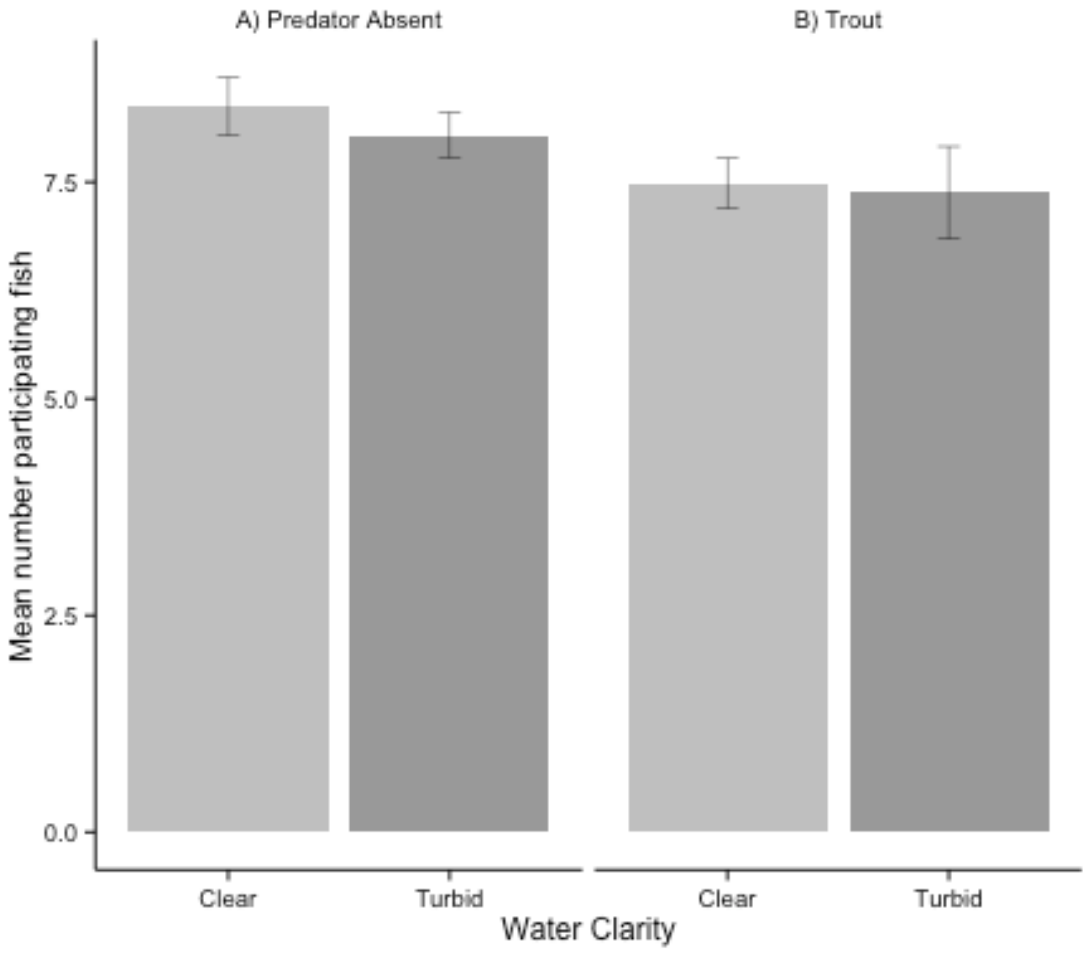


Figure 3-3 Mean participation rate (± 1 SE) of threespine stickleback feeding during trials in clear and turbid conditions with A) trout predator absent, and B) trout predator present.

Chapter 4
General Conclusions

4.1 Thesis summary and findings

In this thesis, I investigated the way in which two previously described, yet relatively under studied, habitat constraints – obstacles to movement between patches, and turbidity – affected patch selection in an aquarium system using the model species threespine stickleback *Gasterosteus aculeatus*. By providing feeding opportunities in a two-chambered aquarium, I was able to introduce habitat variation in a small, simple and easily controlled environment, and study how patch choice differed between treatments. In Chapter 2, I explored how obstacles to movement between food patches affected the ability of stickleback to select between two energetically variable food patches. I showed that altering the number of tunnels available for travel between two food patches caused the fish to switch less often between patches, and thus were less able to gauge and match the resource distribution. However, I did find that groups with moderate travel costs, represented by an intermediate number of tunnels available for travel, most closely matched the predicted distribution. I attributed this finding to the possibility that the stickleback were prioritizing shoal size over energetic gains.

In Chapter 3, I explored whether stickleback use turbid environments to avoid predator detection in a two-factor laboratory experiment, where turbidity, a predator (brook trout *Salvelinus fontinalis*), or both, could be introduced to one chamber of the aquarium. As expected, I saw a reduction in the use of the patch that contained the predator. However, contrary to my prediction, the results also indicated that this species

of stickleback perceives turbidity as a constraint – they tended to avoid feeding in the turbid habitat regardless of level of risk. This result contrasted with a similar experiment performed with fathead minnows *Pimephales promelas*, who preferred the turbid environment even when it contained a predator (Chiu and Abrahams 2010). My results indicate that stickleback are willing to pay an energetic cost of reduced feeding opportunities in order to remain in a clear habitat. I predict that using turbidity as a refuge requires certain adaptations to their physiology, behaviour, or both.

Overall, the results of my thesis demonstrated that when information collection was constrained by physical parameters, the stickleback did not distribute in a manner that maximized energetic gains. There were discrepancies in patch use when both patches were equally rich in resources; and, when patches were unequal, the rich patch was underused and the poor patch was overused – a phenomenon called overmatching. In the experiments studying movement constraints, the results were unclear whether the stickleback were unwilling to pay the energetic cost incurred through known lost feeding opportunities, in order to investigate the second feeder, or if the skewed distribution was due to the fact that food acquisition was not their primary focus at the time.

Habitat selection changes drastically based on current priority (Roever et al. 2014). Animals must frequently trade-off foraging with other behaviours, such as courting/mating (Abrahams 1993), avoiding predation (Abrahams and Dill 1989, Lima and Dill 1990, Houston et al. 1993), caring for offspring (Rachlow and Bowyer 1998), and social behaviour (Webster and Laland 2012). In situations such as these, animals will forgo feeding opportunities, often causing them to move into less energetically rich habitats. This is due, in part, to the fact that resource poor patches are often safer. In both

experiments of this thesis, antipredator behaviour seemed to be of higher priority to the stickleback than food acquisition. The stickleback were collected from an environment containing natural trout predators, which may have been the cause of the shoaling behaviour observed in Chapter 2. In Chapter 3, there was an obvious threat of predation, which was avoided by increasing foraging in the safer chamber.

One could argue that both of the habitat factors studied, turbidity and travel costs, fall under the category of "perceptual constraints", a topic that has been well-studied in regards to habitat selection. Perceptual contraints are one of the violations of the assumptions of the ideal free distribution (IFD) (Fretwell and Lucas 1970). The IFD assumes that all animals have perfect knowledge of the locations of all resources patches, and of the relative profitability of each patch, at all times. Perception constraints describe a situation when animals have a diminished ability to collect information about the resource distribution (Abrahams 1986). While the stickleback used in all of my experiments had been trained to know that a food patch was present in each chamber of the aquarium, both turbidity and travel costs would have diminished their ability to assess the profitability of the patch. The turbidity levels were fairly low, but may have caused enough veiling (Utne-Palm 2002) that the stickleback in the clear chamber were unable to visualize the food entering the turbid chamber, and therefore remained at their starting patch where the resource level was known. Travel constraints diminished the ability of the fish to sample the food patches. It has been reported that sampling is required for this species to properly assess resource profitability (Milinski 1979, Gotceitas and Colgan 1991). As travel between the patches becomes more costly, the fish moved less between the patches. It follows that this may have caused perception constraints at higher travel

costs.

4.2 Future directions

The experiments described in this thesis sought to form a basis of understanding of two specific constraints to habitat selection that aquatic animals encounter. The laboratory setting allowed me to dissect out many of the other factors that come into play during habitat selection in the natural environment, which would have confounded the results.

However, in true habitat selection decisions, countless other biotic and abiotic factors come into play that will alter the way that constraints impact habitat selection. The importance of these constraints may become negligible when we take into account factors such as multiple food sources, competition, and natural predator levels. Additionally, I did not address the fact that these habitat constraints would also impact the behaviour of both the prey, as stickleback feed on zooplankton, and the behaviour of the predators. This introduces a large amount of variability in food web dynamics (Carpenter et al. 1985). A more complex microcosm experiment, similar to those used to simulate climate change effects (Moss et al. 2003, Moran et al. 2010), would be an interesting and viable way to study how turbidity impacts habitat use by those at all three trophic levels, while still limiting the number of other ecological variables.

There remains much to learn about the impacts of constraints on energy flow in the environment. Organisms that occupy a middle trophic level, like planktivorous fish, have enormous impacts on both the higher and lower trophic level species. Any habitat characteristic that alters the distribution of an ecologically important species like the

threespine stickleback (Harmon et al. 2009) can have large cascading effects on the whole community within these areas (Carpenter et al. 1985). Area avoidance that is decoupled from resource distribution can cause overgrazing in the surrounding areas. A modeling exercise that takes into account the physical characteristics like turbidity and areas that would cause movement constraints, would be a useful next step to shed light on the way that these constraints alter energy flow through a system.

4.3 References

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